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**MODELING THE GROWTH KINETICS OF *BACILLUS CEREUS*
AS A FUNCTION OF TEMPERATURE, pH, SODIUM LACTATE
AND SODIUM CHLORIDE CONCENTRATIONS**

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SODYUM LAKTAT VE SODYUM KLORÜR
KONSANTRASYONLARINA BAĞLI OLARAK MODELLENMESİ**

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ABBREVIATIONS

CCD	: Central Composite Design
cfu	: Colony Forming Unit
GR	: Growth Rate
GT	: Generation Time
HACCP	: Hazard Analysis and Critical Control Points
LAG	: Lag Phase Duration
MAFF	: Ministry of Agriculture, Fisheries, and Food
MARE	: Mean Absolute Relative Error
MIC	: Minimum Inhibitory Concentration
MNP	: Most Probable Number
MRE	: Median Relative Error
MSE	: Mean Square Error
MYP	: Mannitol-Egg Yolk –Polymyxin Agar
OD	: Optical Density
PEMBA	: Polymyxin-Pyruvate-Egg Yolk Mannitol-Bromothiol Agar
PM	: Predictive Microbiology
RE	: Relative Error
RMSE	: Root Mean Square Error
RSS	: Residual Sum of Squares
UK	: United Kingdom
USDA-PMP	: United States Department of Agriculture-Pathogen Modeling Program
WHO	: World Health Organization

SYMBOLS

a_w : water activity
 A : asymptotic bacterial count, log(cfu/ml)
 b : regression slope
 B : relative growth rate, h^{-1}
 B_i : parameter coefficients
 C : actual asymptotic amount of growth
 C_i : coefficients
 df : degrees of freedom
 E_a : activation energy, $J\ mol^{-1}$
 k : rate of growth, h^{-1}
 $L(t)$: $\log_{10}$ bacterial count, cfu/ml
 M : time where absolute growth rate is maximum, h
 N : bacterial count at time t , cfu/ml
 N_0 : initial bacterial count, cfu/ml
 NAL : sodium lactate concentration, mM
 $NACL$: sodium chloride concentration, mM
 PH : pH
 q_p : predicted value
 q_o : observed value
 r : growth rate, h^{-1}
 R : gas constant, $J\ K^{-1}\ mol^{-1}$
 t : time, h
 T : Temperature, $^{\circ}C$
 x_p : predicted value
 x_o : observed value
 X : time, h
 y : growth as $\ln(N/N_0)$
 μ_m : maximum specific growth rate, h^{-1}
 λ : lag time, h
 v : equation variable
 τ : equation variable

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MODELING THE GROWTH KINETICS OF *BACILLUS CEREUS* AS A FUNCTION OF TEMPERATURE, pH, SODIUM LACTATE AND SODIUM CHLORIDE CONCENTRATIONS

SUMMARY

Mathematical models describing the growth kinetic parameters (lag phase duration and growth rate) of *Bacillus cereus* as a function of temperature, pH, sodium lactate and sodium chloride concentrations were obtained in this study. f-test was performed to select the most efficient primary level model for describing the change in *B. cereus* numbers with respect to time. According to this, Gompertz function was chosen to calculate the lag phase duration (LAG) and growth rate (GR) of *B. cereus*. Then these kinetic parameters were expressed as a function of the independent variables using the polynomial model and the modified Arrhenius-Davey model. Polynomial models were selected to be used in further study as they have higher R^2 values. In order to obtain a residual distribution closer to a normal distribution, the natural logarithm of the growth kinetic parameters were used in modeling. For reasons of parsimony, the polynomial models were reduced to contain only the coefficients significant at a level of $p \leq 0.05$. Using the reduced polynomial models (R^2 of 95.2 and 93.9 for $\ln$ LAG and $\ln$ GR models, respectively), three dimensional graphs were drawn illustrating the effects of the independent variables on the growth parameters. Predicted data were compared with the predictions from Pathogen Modeling Program (PMP) and validated against data extracted from the literature. The mean absolute relative error (MARE) and the median relative error (MRE) of the predicted generation times with the derived model were 25.6 and -16.7, respectively, showing that the growth rate model accurately and safely predicted the growth kinetics of *B. cereus*. Validation test performed using milk and meat broth also showed that the lag time and growth rate models provide safe estimations. Results of the modeling study indicated that all of the studied factors had a significant affect on the growth of *B. cereus* in the experimental range studied. The antimicrobial activity of sodium lactate was found to be strongly affected by the pH ($p \leq 0.05$) and relatively to a lesser extent by temperature ($p \leq 0.05$). Antimicrobial activity involved both prolonged lag phase duration and reduced growth rate.

Keywords: Predictive Modeling, *Bacillus cereus*, Growth Kinetics, Lag Phase, Growth Rate, Lactate, Temperature, pH, Sodium Chloride.

BACILLUS CEREUS'UN ÇOĞALMA KİNETİĞİNİN SICAKLIK, pH, SODYUM LAKTAT VE SODYUM KLORÜR KONSANTRASYONLARINA BAĞLI OLARAK MODELLENMESİ

ÖZET

Bu çalışmada, sıcaklık, pH, sodyum laktat ve sodyum klorür konsantrasyonlarının *Bacillus cereus*'un çoğalma kinetik parametreleri (lag fazı süresi ve büyüme hızı) üzerindeki etkisini ifade eden matematiksel modeller oluşturulmuştur. *B. cereus*'un zamana bağlı artışını en iyi ifade eden birincil seviye modeli belirlemek için f-testi kullanılmıştır. Buna göre, *B. cereus*'un lag fazı süresi (LAG) ve çoğalma hızını (GR) hesaplamak için Gompertz fonksiyonu seçilmiştir. Daha sonra, bu kinetik parametreler, polinomal ve modifiye Arrhenius-Davey modelleri kullanılarak bağımsız değişkenlere bağlı olarak ifade edilmiştir. R^2 değerleri daha yüksek olduğu için çalışmanın devamında kullanılmak üzere polinomal modeller seçilmiştir. Normal dağılıma daha yakın bir hata dağılımı elde etmek için, modellemede çoğalma kinetik parametrelerinin doğal logaritmaları kullanılmıştır. Bu modeller sadece $p \leq 0.05$ düzeyinde önemli olan katsayıları içerecek şekilde indirgenmiştir. İndirgenen polinomal modeller (ln LAG and ln GR için sırasıyla $R^2 = 95.2$ ve 93.9) kullanılarak, bağımsız değişkenlerin çoğalma parametreleri üzerindeki etkilerini gösteren üç boyutlu grafikler çizilmiştir. Modelin tahminleri, Patojen Modelleme Programının (PMP) tahminleri ile kıyaslanmış ve literatür verilerine karşı doğrulaması yapılmıştır. Oluşturulan model ile hesaplanan jenerasyon sürelerinin ortalama mutlak bağıl hatası (MARE) ve medyan bağıl hatası (MRE) sırasıyla 25.6 ve -16.7 olarak hesaplanmıştır. Bu sonuçlar, çoğalma hızı modelinin *B. cereus*'un çoğalma kinetiğini doğru ve güvenli bir şekilde tahmin ettiğini göstermektedir. Süt ve et suyu kullanılarak yapılan doğrulama testleri de lag fazı süresi ve çoğalma hızı modellerinin güvenli tahminler yaptığını göstermiştir. Modelleme çalışmasının sonuçları, deney sınırları içerisinde, çalışılan tüm bağımsız değişkenlerin *B. cereus*'un çoğalması üzerinde önemli bir etkiye sahip olduğunu göstermiştir. Ayrıca, sodyum laktatın antimikrobiyal aktivitesinin büyük oranda pH'dan ($p \leq 0.05$), nispeten daha düşük oranda sıcaklıktan ($p \leq 0.05$) etkilendiği belirlenmiştir. Sodyum laktatın antimikrobiyal aktivitesi, hem lag fazı süresini uzatma hem de çoğalma hızını azaltma şeklinde ortaya çıkmıştır.

Anahtar Kelimeler: Prediktif Mikrobiyoloji, *Bacillus cereus*, Çoğalma Kinetiği, Lag Fazı, Çoğalma Hızı, Laktat, Sıcaklık, pH, Sodyum Klorür.

1. INTRODUCTION

1. 1. Scope and Objective

Bacillus cereus is an important food borne pathogenic bacteria. Since it is widely distributed in nature, it can be easily found in a wide variety of foods. The WHO (World Health Organization) Surveillance Program for Control of Foodborne Infections and Intoxications in Europe showed that an average of 5-10% of reported foodborne diseases with known etiology were caused by *B. cereus* (WHO Surveillance Programme, 1990). Kramer and Gilbert (1989) and Drobniewski (1993) reported that *B. cereus* accounted for 1 to 3% of the reported outbreaks of bacterial food poisoning in the United States and United Kingdom, 22 % in the Netherlands, 7 % in Canada and 15 % in Hungary between 1970-1980. Such a ratio could not be given for Turkey, since the health statistics are not documented. However, it is not difficult to estimate that similar or even a worse situation is valid for Turkey. Since this microorganism is a common food safety problem, it is worthwhile to obtain a greater understanding about the growth of this organism under various environmental conditions. Such an understanding would enhance our ability to design more effective methods of preservation.

Up to date, the predictive modeling studies on *B. cereus* concentrated mainly on understanding the effects of temperature, atmosphere of the environment, pH and water activity on the growth kinetics of this bacterium (Choma et al., 2000; Chorin et al., 1997; Sutherland et al., 1996; Baker and Griffiths, 1993; Quantivalla and Parolari, 1993). In only one of these modeling studies, the effect of sodium nitrite as a preservative on the growth responses of *B. cereus* was studied (Benedict et al., 1993). However, there exists not much information to predict the specific effects of especially the natural preservatives such as lactate on the growth kinetics of *B. cereus*.

Therefore the main objective of this study was to obtain a mathematical model which quantifies the effects of sodium lactate, sodium chloride, pH and temperature on the growth kinetics of *B. cereus*. In addition, it was also aimed to investigate the

interactions between these selected factors in terms of their effectiveness in inhibiting the growth of the bacterium. Finding out the combinations of the factors that significantly decrease the growth rate or increase the lag time so that a product is safe for a longer period of time was also an expected outcome of this study.



2. THEORY

2. 1. Predictive Microbiology

Predictive microbiology is an alternative way of microbiological analysis. It is based on the premise that since the responses of populations of microorganisms to environmental factors are reproducible, it is possible from past observations to predict the responses of microorganisms. Simply it involves knowledge of microbial growth responses to environmental factors summarized as equations or mathematical models (McMeekin et. al., 1997).

Actually, the subject of predictive microbiology is not new and in fact the calculation of thermal destruction (D-values), botulinum cook studies, and shelf life challenge testing are forms of predictive modeling that began in the 1920's (McNab, 1997; McMeekin et al., 2002). There is a growing interest in quantitative predictive food microbiology because it has many potential applications, such as predicting the effect of changes in product processing or storage conditions, estimating shelf-life, designing, validating, optimizing and controlling production, processing, storage and distribution systems (McMeekin et al., 1993). Moreover, it has considerable potential in providing a cost effective means of food safety risk assessments and for development of HACCP programs (McMeekin and Ross, 2002; McNab, 1997; Soboleva et al., 2000).

Predictive microbiological models can be used to i) predict the microbiological stability of newly developed food products, ii) assess direct and interactive effects of new or different combinations of food preservatives, iii) estimate microbial behavior in foods containing inhibitory factors that could affect microbial growth, iv) to estimate the shelf-life and safety of foods, and vi) to evaluate the potential health hazard of food that has been temperature-abused (Skinner et. al., 1994).

The modeling process has mainly four stages (McMeekin et al., 1993);

- i) Planning
- ii) Data collection and analysis

- iii) Mathematical description
- iv) Validation

2. 1. 1. Planning

2. 1. 1. 1. Design of Experiments

Good quality models can only be developed from high quality data and high quality data come from well-designed experiments. Therefore, it is important to carefully design the experiments in all aspects. The type of design that will be chosen mainly depends on the type of information sought. In general, two types of information can be sought in a study, that is understanding or optimization. In microbial modeling studies, if the main objective is to understand the responses of microorganisms under several influential factors, then factorial design is used to plan the experiments. On the other hand, central composite design (CCD) is best to use if optimization is required, like the optimization of fermentation processes or organic acid production by microorganisms. Another type of design is the screening design, which is used to select the most significant factors before the main experiment are performed (Davies, 1993).

As a rule of thumb, 10-15 data points are required per independent variable to fit most of the kinetic models. There should be more than three values for each variable, and more test conditions than degrees of freedom for the model to be built. The points must be positioned at the areas of inflection where the rate of change of the microbial kinetics is maximal, e.g. at the end of the lag phase and at the end of the logarithmic growth phase (Baranyi and Roberts, 1995; McMeekin et al., 1993; Walker and Jones, 1993).

2. 1. 1. 2. Preparation of Inocula

In order to be able to make conservative predictions, it is suggested to use a cocktail of strains in the experiments. A typical cocktail will contain three and five strains (Walker and Jones, 1993). Using mixture of strains is advantageous in that, it is more representative of real food situations (Gibson et al., 1987; Gibson et al., 1988). On the other hand, the use of a single strain inoculum may be easier to model and shows how a single strain behaves over a wide range of environmental conditions (Davies, 1993). Walker and Jones (1993) stated that an alternative is to model

several strains of the same microorganism and to choose the strain which grows fastest under the conditions of most interest for further study.

The physiological state of the bacterial cells may have an effect on the subsequent growth kinetics, especially on the lag phase duration. Therefore for obtaining comparable results, it is important to use physiologically homogeneous inocula. One way to ensure that is to use exponential phase cells, commonly named as 'overnight culture' in the literature (Walker et al., 1990; Buchanan and Klawitter, 1991).

Another important thing about the inocula is to use enough high levels of initial inoculum, so that the detection time of the measurement method can be distinguished from the lag time of the microorganisms. If the initial inoculum level used is below this level then the time taken for an observable change in bacterial number will be a combination of the lag time and the time taken for the population size to increase to a detectable level (Buchanan and Phillips, 1990). But, it is also important that the inoculum size be low enough to allow growth to be measured before the microorganisms change the environment. Typically an initial inoculum level of 10-1000 cfu/ml is recommended for growth studies (Walker and Jones, 1993).

Using real foods in modeling studies is costly, logistically very difficult, and in general limits the method of measurement to viable counts or most probable number (MNP) methods. For this reason, liquid media, which allows a greater range of detection methods, is preferred in predictive microbiology studies. But a reasonable criticism of liquid media is that they may not accurately model conditions in real foods, so that the model will subsequently have to be validated in the food product to which it is intended to be applied (Ross and McMeekin, 1991).

2. 1. 2. Data Collection and Analysis

Microbial enumeration methods may be classified as direct in which individual organisms are distinguished and counted (e.g. direct microscopy, coulter counter, plate counts), or indirect in which some property of the total population (e.g. DNA, dry weight, impedance, optical density) is assayed. In indirect methods, a calibration is prepared to relate cell density to the level of the measured property (McMeekin et al., 1993). The literature survey on predictive microbiology showed that mostly viable (plate) count methods or optical density determinations are used in microbial modeling.

Advantages of plate count methods are high sensitivity, measurement of only viable cells, and requirement of low capital investment. However, they require high labor input, high amount of consumables only for a single determination, have relatively low reproducibility and a long elapse time (Mattila, 1987).

Alternatively, turbidometry is a rapid, non-destructive and inexpensive method for monitoring the growth of microbial cultures. However turbidometry is limited to measuring growth only in clear liquid media. In addition, for measurements on dense cultures, there is a deviation from the response predicted by Beer's Law resulting in falsely low estimates of cell density. To overcome this problem, it is necessary to dilute the samples to achieve absorbance < 0.3 , or that the observed absorbance be 'corrected' by reference to an appropriate correction function. Another difficulty in using turbidometry is the lower sensitivity limit of turbidity measuring devices. In general, they are unable to detect populations of less than about 10^6 cfu/ml. Given that the upper limit of sensitivity is of the order of $10^{7.5}$ cfu/ml, and that under conditions permitting consistent growth the stationary phase is typically of the order of 10^9 cfu/ml, the onset of stationary phase is not easily measurable, and the lag time determinations can only be made on dense populations (McMeekin et al., 1993).

2. 1. 3. Modeling

Microbial modeling is the use of mathematical expressions to describe microbial behavior. The nature of organism of concern and the problem it causes will partly determine the type of model required (McMeekin et al., 1993). Models can be classified by the microbiological event studied, the modeling approach used, or the variables considered. A three level classification method described as primary, secondary and tertiary models was proposed by Whiting and Buchanan (1994) for microbial models.

2. 1. 3. 1. Primary Level Models

Primary level models describe how microbial numbers change with time in a specific environment. These include growth and inactivation/survival models, Production of metabolic products (toxins, substrates) also can be modeled. Mainly two approaches is used in primary modeling: models based on probability of growth and models that describe the kinetics of growth. Kinetic models have been developed for vegetative pathogens (Whiting and Buchanan, 1994).

Probability models have mainly been developed for *Clostridium botulinum*, where any growth accompanied with toxin production is considered hazardous (Hauschild, 1982; Skinner and Larkin, 1994). Simple probabilistic models are relatively simple, easy to use and quickly give an indication about the risk. However, the amount of information obtained from such equations is limited. Moreover, these models are not always precise and may be influenced by trends in the data. Nevertheless, Roberts (1989) stated that simple probability models are first attempts to predict risks associated with foods.

An alternative approach to understanding the microbial response in foods is to model the growth kinetics, particularly the lag time and the specific growth rate. The lag time is important because it indicates the maximum time before growth will occur. The 'specific growth rate' is useful because it can be used for predictions irrespective of the assumed initial contamination level, or the amount of increase predicted, and can be measured in most cases before growth begins to alter the substrate under test (Baird-Parker and Kilsby, 1987).

There are five sigmoidal functions used to model microbial growth: Logistic (Gibson et al., 1987), Gompertz (Gompertz, 1825), Richards (Richards, 1959), Stannard (Stannard et al., 1985), and Schnute (Schnute, 1981) functions. The common point of these five models is that, they describe the number of microorganisms and do not include the consumption of substrate as a model based on the Monod equation would do (Zwietering et al., 1990).

The logistic function is given in Eq. (2. 1),

$$y = a / (1 + \exp(b-cx)) \quad (2. 1)$$

where y is growth ($\ln(N/N_0)$), N is bacterial number in cfu/ml, x is time, and a, b and c are mathematical parameters.

The Gompertz function is given in Eq. (2. 2),

$$y = a \exp(-\exp(b-cx)) \quad (2. 2)$$

where the variables have the same meaning as given for the logistic function [Eq. (2. 1)].

The Gompertz function can also be written as follows:

$$L(t) = A' + C \exp(-\exp(-B(t - M))) \quad (2.3)$$

where $L(t)$ is $\log_{10}$ bacterial count (cfu/ml) at any time t , A' is the asymptotic count as t decreases to zero (i.e., initial level of bacteria, N_0), $A'+C$ is the asymptotic number of microorganisms present as t increases indefinitely with C being the actual asymptotic amount of growth, and B is the relative growth rate at M , where M is the time where the absolute growth rate is maximum (Gibson et al., 1987).

Using Equation 2.3, the growth rate (GR), the generation time (GT) and the lag phase duration (LAG) can be calculated according to the following equations (McMeekin et al., 1993):

$$GR = B \cdot C / e \quad (e = 2.718) \quad (2.3.a)$$

$$GT = \log_{10} 2 / GR \quad (2.3.b)$$

$$LAG = M - (1/B) \quad (2.3.c)$$

As Gompertz model, most of the equations describing a sigmoidal growth curve contain mathematical parameters (a , b , c) rather than parameters with a biological meaning (A , λ , μ_m). A is the maximal value reached ($\ln(N_\infty/N_0)$); the maximum specific growth rate, μ_m is defined as the tangent in the inflection point; the lag time, λ is defined as the x-axis intercept of this tangent. It is difficult to estimate start values for the parameters if they have no biological meaning. Therefore, models can be rewritten to substitute the mathematical parameters with A , λ , and μ_m . As an example, we show here the modification of the Gompertz equation, which is rewritten as (McMeekin et al., 1993; Zwietering et al., 1990):

$$y = a * \exp[-\exp(b-ct)] \quad (2.4)$$

The second derivative of the function with respect to t gives the inflection point of the curve:

$$dy/dt = ac * \exp[-\exp(b-ct)] * \exp(b-ct) \quad (2.4.a)$$

$$d^2y/dt^2 = ac^2 * \exp[-\exp(b-ct)] * \exp(b-ct) * [\exp(b-ct)-1] \quad (2.4.b)$$

At the inflection point, where $t=t_i$, the second derivative is equal to zero:

$$d^2y/dt^2 = 0 \quad t_i = b/c$$

The expression for maximum specific growth rate can be derived by calculating the first derivative at the inflection point.

$$\mu_m = (dy/dt)_{t_i} = ac/e \quad (e = \exp(1)) \quad (2.4.c)$$

The parameter c in the Gompertz equation can be substituted for by;

$$c = \mu_m e/a \quad (2.4.d)$$

The description of the tangent line through the inflection point is:

$$y = \mu_m t + a/e - \mu_m t_i \quad (2.4.e)$$

The lag time is defined as the t -axis intercept of the tangent through the inflection point:

$$0 = \mu_m \lambda + a/e - \mu_m t_i$$

Using equations 4, 5, and 7 yields:

$$\lambda = (b-1)/c \quad (2.4.f)$$

The parameter b in Gompertz equation can be substituted by:

$$b = (\mu_m e/a) \lambda + 1 \quad (2.4.g)$$

The asymptotic value is reached for t approaching infinity:

$$t \rightarrow \infty : y \rightarrow a \Rightarrow A = a$$

The parameter a in the Gompertz equation can be substituted for by A , yielding the modified Gompertz equation:

$$y = A \exp\{-\exp[(\mu_m e/A)(\lambda - t) + 1]\} \quad (2.5)$$

The sigmoidal Richards (1959) equation, as given by Zwietering (1990) is shown below [Eq. (2.6)].

$$y = a (1 + v \exp(k(\tau - x)))^{-1/v} \quad (2.6)$$

where y is growth ($\ln(N/N_0)$), x is time, and a , v , τ and k are equation variables. Richards equation is an extension of the sigmoidal growth function of von Bertalanffy (1957), which was intended to model the growth of animals.

Stannard et al. (1985) used the following sigmoidal function to represent the growth curves of certain psychrotrophic food spoilage bacteria [Eq. (2.7)].

$$y = A / (1 + \exp(-(1 + kx)/p))^{(-p)} \quad (2.7)$$

where y is microbial growth, x is time, and A , k , and p are the parameters of the curve. This equation was found satisfactory in fitting growth curves of pure cultures and binary mixtures of the psychrotrophic microorganisms (Skinner et al., 1994).

Schnute (1981) developed a comprehensive sigmoidal function to fit the growth behavior of fish, but it may be extended to other organisms (Skinner et al., 1994).

$$Y = \{y_1^b + [y_2^b - y_1^b] \times [(1 - \exp(-a(t - \tau_1))) / (1 - \exp(-a(\tau_2 - \tau_1)))]\}^{1/b} \quad (2.8)$$

where Y represents fish size or some aspects of fish growth at time t , and b is a dimensionless constant defined by Schnute (1981) as the relative incremental rate of growth, relating the maximum and minimum size values of the sigmoidal growth curve of fish as a function of time; a is the constant relative rate of growth associated with the size difference b , τ is fish age, and y is fish size at age τ .

Among the five models, Zwietering et al. (1990) concluded that the Gompertz function was the best model for describing the growth data tested, noting that if a three parameter model sufficiently describes the data, it is recommended, because

of its simplicity, over a four or more parameter model. The two most commonly used sigmoidal functions are the Gompertz and logistic functions (Skinner et al., 1994). Sigmoidal functions are useful because actual microbial growth rate is not constant over the entire growth period, but rather it increases to a maximum then decreases (Gibson et al., 1987). Both the Gompertz and logistic functions do not assume constant growth rate (Garthright, 1991).

2. 1. 3. 2. Secondary Level Models

Secondary level models indicate how parameters (e.g. Gompertz A, C, B, and M) of primary models change with respect to one or more environmental or cultural factors (e.g., atmosphere, pH, temperature, and salt level) (Whiting and Buchanan, 1994).

Secondary models have generally been of three types: response surface equations (multiple polynomial regression equations), the Arrhenius relationship, and the square root (Bélehrádek) models (Ross and McMeekin, 1994; Whiting and Buchanan, 1994).

The simplest form of 'Arrhenius-type' model in use in predictive microbiology is the 'classical' Arrhenius equation [Eq. (2. 9)] (Ross and McMeekin, 1994).

$$\ln k = \ln A - E_a / RT \quad (2. 9)$$

where, when applied to microbial growth, k is the rate of growth, A is a parameter to be fitted, R is the gas constant (8.314 J K⁻¹ mol⁻¹), T is temperature, and E_a is referred to as a 'temperature characteristic', or the activation energy of the growth-rate-limiting reaction. Equations in this group are based on an assumed exponential relationship between temperature and reaction rate (Skinner et al., 1994). Modified versions (Davey, 1989, 1991) of the Arrhenius equation include the effects of a_w and pH, also [Eq. (2. 10)].

$$\ln k = C_0 + C_1 / T + C_2 / T^2 + C_3 a_w + C_4 a_w^2 \quad (2. 10)$$

where k is the growth rate, T is temperature, a_w is the water activity, and C₀, C₁, C₂, C₃, C₄ are coefficients to be determined. Davey (1989) used the Arrhenius approach to develop a modified or additive Arrhenius model for predicting the combined effects of temperature and a_w on bacterial growth rate. He stated that this equation

is referred to as additive because it contains no interaction term between temperature and water activity.

Square-root models were originally based on the observation of Ohta and Hirahara (1977) that the square root of the rate of nucleotide degradation in carp muscle is related to temperature. In 1986, Olley found that this relationship well describes the growth rate response to temperature for many bacteria. Subsequently it was realized that the 'square-root' model was a special case of the Bélehrádek (1930) equation widely used in other biological sciences (Ross, 1987). The equation has the form:

$$\sqrt{r} = b (T - T_0) \quad (2. 11)$$

where r is growth rate, b is the regression slope, T is temperature, and T_0 is a conceptual temperature below which the growth rate is zero and the lag time is infinite (Skinner et al., 1994).

Ratkowsky et al. (1983) recognized that Eq. (2. 11) was limited to growth temperatures at and below optimal growth and extended the applicability of equation [Eq.(2. 12)] to predict growth at any temperature.

$$\sqrt{r} = b (T - T_0) \times (1 - e^{(c(T - T_{max})))} \quad (2. 12)$$

where T_{max} is a conceptual maximum temperature at which the rate of growth is zero, and b and c are regression coefficients.

McMeekin et al. (1987) further extended the general form to include a term for water activity [Eq.(2. 13)].

$$\sqrt{r} = b (T - T_0) \sqrt{(a_w - a_{wmin})} \quad (2. 13)$$

where a_w is the water activity and a_{wmin} is a notional minimum water activity for growth.

After this, Adams et al. (1991) incorporated a term for pH and obtained the below form [Eq.(2. 14)]:

$$\sqrt{r} = b (T - T_0) \sqrt{(pH - pH_{min})} \quad (2. 14)$$

where pH_{min} is a notional minimum pH for growth. The success of these two latter equations led McMeekin et al (1992) to modify the equations to further include the effects of temperature, pH, and water activity in one model as given in Eq.(2. 15).

$$\sqrt{r} = b (T - T_0) \sqrt{(a_w - a_{wmin}) (pH - pH_{min})} \quad (2. 15)$$

where all parameters have the same meanings as previously defined. This equation successfully described the growth of *Listeria monocytogenes* (Wijzes et al., 1993).

Response surface models, which were developed originally for process optimization, represent a purely empirical approach to the problem of summarizing growth rate responses. In this technique a linear model is constructed, which has the form of a polynomial function in the modeled parameters. Multiple linear regression is used to determine the best fit values for the parameters. The regression equations have the general form [Eq.(2. 16)] (Ross and McMeekin, 1994):

$$Y = a + b_1X_1 + b_2X_2 + \dots + b_iX_i + \dots + b_nX_1^2 + \dots + b_i^1X_i^2 + \dots + b_{2i}X_i X_j \quad (2. 16)$$

where $a, b_{1,2,\dots,z}$ are parameters to be estimated and $X_{1,2,\dots,i,j}$ are variables.

2. 1. 3. 3. Tertiary Level Models

Tertiary level models assist in the use of primary and secondary level models by identifying and using pertinent information to calculate and display desired graphs, predictions and comparisons. Examples are mathematical expressions, software packages, and expert systems (Whiting and Buchanan, 1994).

Mainly two tertiary level models are available now. The USDA-PMP (U.S. Department of Agriculture-Pathogen Modeling Program), and the MAFF-Food MicroModel of the UK Ministry of Agriculture, Fisheries, and Food. These programs provide a user-friendly format for application of available response surface models for broth cultures of *Aeromonas hydrophila*, *B. cereus*, *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Shigella flexneri*, *Staphylococcus aureus* and *Yersinia enterocolitica* (Buchanan, 1993; Whiting and Buchanan, 1994).

2. 1. 4. Validation of models

The ultimate success of a predictive model is judged by its efficiency in predicting the response of microorganisms in real food systems. Therefore, before a model is used, it should be validated using extra data extracted from the literature (McMeekin et al., 1993; Baranyi et al., 1996; Oscar, 1999).

In validation, the reliability of the model predictions can be assessed by quantifying the accuracy and bias of the model predictions. The median relative error (MRE) and the mean absolute relative error (MARE) of model predictions are used as a measure of the prediction bias and the accuracy of the model, respectively. In calculating these two parameters relative error (RE) is found using the following equation:

$$RE = (x_p - x_o) / x_o \quad (2. 17)$$

where x_p is the predicted value and x_o is the observed value.

Then the MARE is calculated according to the following equation:

$$MARE = (1/n) \sum |RE_i| \quad (2. 18)$$

where n is the number of cases (Oscar, 1999; Delignette-Muller et al., 1995).

2. 1. 5. Selection of Models and Goodness of Fit

Ratkowsky et al. (1991) stated that the mean square error (MSE) alone is shown to be an inappropriate criterion by which to compare the performance of predicted models with different number of parameters. One way to discriminate among such models is to compare them statistically by the use of F test. In this test, the difference between the RSS values for the models is compared to the RSS of a general model. The following is then calculated:

$$f = [(RSS_2 - RSS_1) / (df_2 - df_1)] / (RSS_1 / df_1) \text{ tested against } F(\alpha, df_1, df_2 - df_1) \quad (2. 19)$$

where RSS_1 is the RSS from the general model with a degrees of freedom of df_1 , and RSS_2 is the RSS from the model to be judged with a degrees of freedom of df_2 . If the f value is smaller than the table F value, then the model is statistically

acceptable. After the selection of all the statistically acceptable models, other criteria could be used to choose the best model. First the models with the fewest number of parameters are selected, then from this subset, the model with the lowest RSS is selected (Zwietering et al., 1990).

The goodness of fit of the polynomial equations to the data can be judged by root mean square error (RMSE); the lower the value of the RMSE, the better the fit (Sutherland et al., 1994). Fernandez et al. (1997) and Sutherland et al. (1994) used the RMSE to assess the performance of a model as follows:

$$\ln q_{\text{obs}} - \ln q_{\text{pred}} \approx \text{RMSE} \quad (2. 20)$$

where q_{obs} is the observed and q_{pred} is the predicted value.

Transforming it gives the below:

$$\ln(q_{\text{obs}}/q_{\text{pred}}) \approx \text{RMSE} \quad (2. 20. a)$$

$$q_{\text{obs}}/q_{\text{pred}} \approx \exp(\text{RMSE}) \quad (2. 20. b)$$

$$(q_{\text{obs}}/q_{\text{pred}}) - 1 \approx \exp(\text{RMSE}) - 1 \quad (2. 20. c)$$

$$(q_{\text{obs}} - q_{\text{pred}})/(q_{\text{pred}}) \approx \exp(\text{RMSE}) - 1 \quad (2. 20. d)$$

The R^2 statistic is often used as an overall measure of the fit attained. It measures the fraction of the variation about the mean that is explained by the fitted model (Box and Draper, 1987). The higher the value, the better is the fit of the data to the model.

2. 2. *Bacillus Cereus*

Bacillus cereus, which is a member of the bacterial genus *Bacillus*, is a Gram-positive, spore-forming, mobile, aerobic, rod shaped bacterium (Granum, 1994; Drobniewski, 1993; Dufrenne et al., 1995). *B. cereus* is widely distributed in nature. It is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water, and sediments (Kramer and Gilbert, 1989). Therefore it can easily be found in a variety of foods, including milk and dairy products, meats, spices, dried products,

cereals, especially rice and eggs (Kramer and Gilbert, 1989; Roberts et al., 1996) and also from drugs including oral pharmaceutical products (Arribas et al., 1988). Since the spores of this microorganism are heat resistant and therefore generally survive cooking, they may also be present in processed foods (Granum, 1994). The organism is not a hazard at the very low levels normally present in foods. However, problems develop when especially cooked foods, which are devoid of most heat sensitive microbial competitors, are held at 10-55°C for a long period of time. Under these conditions, the organism grows to large numbers, releasing toxin during growth in the food and in the intestinal tract following ingestion (Doyle, 1988).

2. 2. 1. Isolation and Identification

Isolation of *B. cereus* from food involves plating on differential and selective media which contain selective agents to inhibit competitive microorganisms and facilitate enumeration. Many different plating media have been developed, based on the principle of providing conditions permitting the organism to exhibit haemolysis production, lechitinase activity, fermentation properties or morphological features characteristic of the species (Roberts et al., 1996).

The primary selective plating media includes polymyxin B as an inhibitory agent for competitive organisms and are designed to use the mannitol negative, lecithin-hydrolyzing nature of *B. cereus* for differentiation (Meer et al., 1991). The media most commonly used are mannitol-egg yolk-polymyxin agar (MYP) (Mossel et al., 1967) or polymyxin-pyruvate-egg yolk mannitol-bromothylol blue agar (PEMBA) (Holbrook and Anderson, 1980). Typical *B. cereus* colonies on the PEMBA plates are peacock blue with a surrounding zone of precipitation. On MYP, colonies typical of *B. cereus* have a dry, flat, ground glass appearance that may be translucent to creamy-white with a violet-red background surrounded by a readily visible zone of egg yolk precipitate (Meer et al., 1991).

Since very few organisms produce similar reactions on egg yolk-polymyxin-containing media to those elicited by *B. cereus*, differentiation from non-Bacillus species is rarely required. Specific identification of *B. cereus* requires differentiation from *Bacillus mycoides* (on the basis of colonial morphology) (Rajkowski and Mikolajcik, 1987), *Bacillus thuringiensis* (*B. cereus* lacks a parasporal insect-toxic crystal), and *Bacillus anthracis* (*B. cereus* is motile). Various biochemical tests can be used, but none of them are as rapid or effective as the colonial and microscopic examinations (Lancette and Harmon, 1980; Kramer and Gilbert, 1989).

2. 2. 2. Pathogenity

Clinical infections caused by *B. cereus* fall into six broad group: (i) local infections, particularly of burns, traumatic or postsurgical wounds, and the eye, (ii) bacteremia and septicemia, (iii) central nervous system infections, including meningitis, abscesses, and shunt-associated infections, (iv) respiratory infections, (v) endocarditis and pericarditis, (vi) food poisoning, characterized by toxin-induced emetic and diarrheagenic syndromes (Dobrowniewski, 1993).

B. cereus is a significant cause of food-related diseases, the incidence of which varies appreciably around the world. Many strains of *B. cereus* elaborate a variety of extracellular metabolites, including toxins and virulence factors. These metabolites are produced mainly during the exponential phase of growth. *B. cereus* produce seven different toxins which can be divided into four groups: (i) diarrhoeal enterotoxin, (ii) haemolysins (cereolysin, haemolysin II, sphingomyelinase, (iii) phospholipase C (phosphatidylinositol hydrolase, phosphatidylcholine hydrolase, sphingomyelinase), (iv) emetic toxin. The emetic toxin and the enterotoxin is important from the food safety point of view (Granum, 1994; Drobniewski, 1993; Roberts et al., 1996).

The emetic toxin or vomiting factor is responsible for the emetic outbreaks characterized by nausea and vomiting within 0.5 to 6 h after ingestion of contaminated food (Meer et al., 1991). It has been suggested that the emetic toxin may be a breakdown product from foodstuffs supporting the growth of *B. cereus* (Granum, 1994). Production of the toxin occurs at mesophilic temperatures (25-30°C) during the stationary growth phase (Meer et al., 1991). It is a small peptide with a molecular weight of <5000. The toxin is extremely heat stable, withstanding a temperature of 126°C for 90 min (Keay and Wildi, 1970). Moreover the toxin is stable in the pH range of 2 to 11 (Roberts et al., 1996; Meer et al., 1991). Emetic outbreaks seem to be almost entirely associated with rice (Meer et al., 1991).

The enterotoxin or the diarrhoeagenic factor is a cytotoxic (membrane disrupting) protein with a molecular weight of about 38.000-46.000 (Granum et al., 1993; Roberts et al., 1996). This heat labile enterotoxin, which can be inactivated by exposure to 56°C for 30 min (Roberts et al., 1996), is produced and released during the late exponential phase of growth (Dufrenne et al., 1994). It can be produced in the temperature range of 18 to 43°C and at pHs between 4 to 11 (Meer et al., 1991). It causes the onset of watery diarrhea and abdominal cramps occurring 5 to 6 h after

consumption of the contaminated food. Enterotoxin production is hard to detect before *B. cereus* has reached 10^7 cells / ml. However, the total infective dose of *B. cereus* is in the range of 10^5 - 10^8 cells (Kramer and Gilbert, 1989)

2. 2. 3. Growth and Survival Characteristics

There are mainly two types of *B. cereus* strains: psychrotrophic and mesophilic. Most of the psychrotrophic strains isolated from food stored at refrigerator temperatures (Christiansson et al., 1989; Van Netten et al., 1990) have been reported to have a minimum growth temperature of 7°C (Rajkowski and Mikolajcik, 1987; Dufrenne et al., 1994), 6°C (Granum et al., 1993) or even 4-5°C (Meer et al., 1991), but can not grow at 30-35°C (Roberts et al., 1996). These strains are of increasing concern to the food industry in producing ready to eat and fresh chilled foods. Mesophilic strains of *B. cereus* can grow between 15-55°C, the optimum growth temperature being in the range of 30-40°C (Roberts et al., 1996). The minimal pH for growth also varies depending both on the type of strain and the acidulant used. In general, growth does not occur at pH 4.8 in media acidulated with HCl, or at pH 5.6 in media acidulated with lactic acid (Roberts et al., 1996). The minimum water activity level for *B. cereus* growth is reported to be 0.93 (ICMFS, 1996). The dependence of *B. cereus* growth on water activity is not well documented, but it is known that depending on the humectant, the minimum for water activity changes. The bacterium will not grow at a_w 0.92-0.93 when NaCl is used as the humectant (Marshall et al., 1971; Measures, 1975); on the other hand it can grow at a_w 0.93 but not at a_w 0.92 in media containing glycerol as the humectant (Jacobsen et al., 1972; Marshall et al., 1971).

2. 2. 4. Modeling Studies

Up to date both turbidometric (Quintavalla and Parolari, 1993; Baker and Griffiths, 1993; Chorin et al., 1997; Choma et al., 2000) and plate count techniques (Benedict et al., 1993; Sutherland et al., 1996) were noted to be used for monitoring the growth of *B. cereus* in modeling studies. Sutherland et al. (1996), Baker and Griffiths (1993), Chorin et al. (1997) and Quintavalla and Parolari (1993) used second order polynomial equations to model the growth kinetic parameters depending on the independent variables. Quintavalla and Parolari (1993) and Baker and Griffiths (1993) defined growth as time required to reach a predefined level of cell concentration, whereas Chorin et al. (1997) used Gompertz function to calculate the

growth rate and lag time, and Sutherland et al. (1996) used Baranyi model (Baranyi et al., 1993) to model the bacterial numbers as a function of time and to calculate doubling times for *B. cereus*.

In these modeling studies on *B. cereus*, data were mainly concentrated on temperature, pH, water activity and sodium chloride concentration, the variable ranges for which is given in Table 2.1. (Choma et al., 2000; Baker and Griffiths 1993; Chorin et al. 1997; Quintavalla and Parolari, 1993). Other than these, Sutherland et al. (1996) studied the effect of CO₂ concentration in combination with temperature, pH and NaCl concentration on the doubling time of *B. cereus* and Benedict et al. (1993) studied the effect of various sodium nitrite concentrations under variable temperature, pH and NaCl concentrations on the lag time, generation time and maximum population density of *B. cereus* (Table 1).

Table 2. 1. Modeling studies for *B. cereus*.

Reference	Variables and ranges
Choma et al., 2000	T (5-38°C)
Chorin et al., 1997	T (7-30°C); pH (5.0-7.5); a_w (0.991-0.95)
Sutherland et al., 1996	T (10-30°C); pH (4.5-7.0); NaCl (0.5-10.5 %w/v); CO ₂ (0-80 %v/v)
Benedict et al., 1993	T (5-42°C); pH (4.5-7.5); NaCl (0.5-5.0 %); NaNO ₂ (0-200 ppm)
Baker and Griffiths, 1993	T (6-38°C); pH (5.8-8.0); a_w (0.965-0.995); Glucose (0-1.8 %); Starch (0-0.625 %)
Quintavalla and Parolari, 1993	T (11.5-28.5°C); pH (4.2-7.0); a_w (0.91-0.96)

2. 3. Lactates

Lactic acid is one of the most widely distributed acids in nature, and together with acetic acid is the most widely employed as preservative. The sodium, potassium and calcium salts of lactic acid are approved for use in foods as direct ingredients (Shelef, 1994). Sodium and potassium lactate are generally available as 60% aqueous solution with a neutral pH. They are used as humectants and flavor enhancer in meat and poultry products and contribute to increased cooking yield and water holding capacity (Duxbury, 1988). Calcium lactate is a hygroscopic powder available in the monohydrate form. It is mainly used for dietary calcium

supplementation, prevention of ropiness in bread dough, firming agent and gel production from demethylated pectins (Shelef, 1994).

Among these three lactic acid salts, sodium lactate is preferred to be used in meat products due to several reasons. First of all, addition of sodium lactate has no influence on the pH. It has a mild salty taste in comparison with sodium chloride and its natural occurrence in meat makes it a suitable flavoring agent (de Raat and van Burik, 1990). Furthermore, sodium lactate has been reported to exhibit antioxidant activity (Nnanna et al., 1994) and has a shelf-life prolonging effect (Wit and Rombouts, 1990; Houtsma et al., 1993). Moreover sodium lactate has an antimicrobial activity which depends on the acid concentration, temperature, and the type of organism (Greer and Dilts, 1992). It has also been reported that it delays toxin production by *Clostridium botulinum* in cooked-in-bag-turkey products (Maas et al., 1989).

Up to date studies have proven that the specific inhibitory effect of sodium lactate on various microorganisms evidently depends on how these microorganisms can cope with this compound (Houtsma et al., 1996; de Wit and Rombouts, 1990). In general, several possible mechanisms have been proposed for the antimicrobial activity of lactates: lowering of intracellular pH, lowering water activity, inhibition of ATP formation, chelating with iron, and inhibition of enzymes involved in the pyruvate-to-lactate conversion (Papadopoulos et al., 1991).

Houtsma et al. (1996) reported that the temperature does not have a specific influence on the minimum inhibitory concentration (MIC) values for sodium lactate for most of the bacteria, except for *Debaryomyces hansenii*. Moreover, in general if MIC values of sodium lactate decreased at low temperature, those of NaCl decreased as well. Compared to NaCl, much lower concentrations of sodium lactate were needed to prevent growth. On the other hand, de Wit and Romboust (1990) reported that adverse growing conditions, such as suboptimum temperature, may increase the antimicrobial effect.

Moreover, for most of the bacteria including *Brochothrix thermosphacta*, *Carnobacterium piscicola*, *Listeria innocua*, *Listeria monocytogenes*, *Pseudomonas fragi*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Yersinia enterocolitica*, MIC values of sodium lactate strongly decreased at low pH values. For most of them the MIC value of sodium lactate was higher than 893 mM at a pH of 7.0, and reduced to lower than 268 mM at a pH of 5.7. However, for *Staphylococcus aureus*, *Lactococcus cremoris*, *Lactococcus lactis* and some lactic acid bacteria isolated

from spoiled meat products, the MIC value of sodium lactate almost did not change with pH. The MIC values of NaCl appeared to be independent of the pH in the range 5.7 to 7.0 for all microorganisms that were screened (Houtsma et al., 1996).

Grau (1980 and 1981) and Young et al. (1993) showed in their studies that the pH has a more pronounced effect on the sensitivity of microorganisms to sodium lactate than temperature has, and that the inhibition by sodium lactate is not only due to osmotic or sodium ion effects. This specific pH effect is explained by the fact that sodium lactate is a weak organic acid and that the lower the pH, the higher is the concentration of undissociated acid in the medium. The diffusion of the undissociated acid molecule across the plasma membrane, results in a lowering of the intracellular pH (Salmond et al., 1984; Cherrington et al., 1991; Shelef, 1994). When the intracellular pH of a microorganism decreases below a certain threshold value, cellular functions are inhibited (Kashket, 1987). Therefore for a large number of bacteria that were screened the antimicrobial effect of sodium lactate increased with decreasing pH values and became most pronounced at acidic pH values (Houtsma et al., 1996; Murphy et al., 1997; Blom et al., 1997). On the other hand, Russel (1992) showed that some bacteria have the ability to maintain a more or less constant pH gradient over the cell membrane instead of keeping the intracellular pH at a high level. These bacteria may be able to avoid the accumulation of high and potentially toxic concentrations of lactate anions at low pH levels. Even in the absence of weak organic acids, it may be assumed that at lower extracellular pH values, bacteria will be stressed as they have to keep their intracellular pH sufficiently high (Houtsma et al., 1996).

Another explanation for the different response of various microorganisms to lactate may be that the lactate uptake in certain microorganisms could be carrier mediated. In this type of transport, stereospecific, membrane-located carriers allow a specific compound to diffuse through the membrane which otherwise are impermeable to the membrane. This type of transport is found for the transport of glycerol in a number of species of *Bacillus*, *Pseudomonas*, *Escherichia coli*, *Shigella*, and *Klebsiella* (Neidhardt et al., 1990). Carrier mediated transport is fully reversible and the driving forces (proton gradient, lactate gradient) determine which way the transport will be (Ten Brink et al., 1985). Accumulation of lactate may be counteracted by carrier mediated efflux. Both of the process will normally require energy, which cannot be spent on synthesis and growth. This loss of metabolic energy results in reduced growth rate and prolonged lag phase as observed by de Wit and Rombouts (1990) for *Streptococcus faecalis*, *Staphylococcus aureus*, and *Salmonella typhimurium*.

Water activity lowering capacity may be another reason for the inhibitory effect of sodium lactate. Chirife and Fontan concluded that (1980) sodium lactate does not present any exceptional water activity lowering property and is definitely inferior to sodium chloride for depressing water activity at equal weight concentrations. Similarly, de Wit and Romboust (1990) stated that the antimicrobial effect of sodium lactate cannot be ascribed to mainly to its water activity lowering effect and the specific inhibitory effect of sodium lactate on various microorganisms evidently depends on how these microorganisms can cope with this compound. They found that at same a_w levels, the growth of *Streptococcus faecalis*, *Staphylococcus aureus*, and *Salmonella typhimurium* inhibited more by sodium lactate than by sodium chloride in the same growth medium. However, the growth rate of *Escherichia coli* is hardly affected by even 5% sodium lactate. It was seen that equal molar concentrations of sodium lactate and NaCl ranging from 0 mM to 893 mM cause an almost equal reduction of a_w and moreover, at these levels the MIC values for sodium lactate are always lower than those for sodium chloride which reflect the specific inhibitory effect of sodium lactate on microorganisms, except for *B. cereus* strains which are found to be sensitive to almost equal concentrations (600-800 mM, $a_w=0.976-0.973$) of sodium lactate and NaCl (Houtsma et al., 1993).

Shelef (1994) reported that in broth containing 0.3% sodium lactate, the growth of *B. cereus*, *Bacillus subtilis* and *Bacillus circulans* were suppressed, whereas 12% of the salt was needed for complete inhibition. Aran (2001) found that the inhibitory effect against *B. cereus* was evident in the presence of 3% sodium lactate in beef goulash.

In complimentary studies in foods, effective inhibition of *Listeria monocytogenes* was obtained for up to 4 weeks by incorporating 2% (w/w) sodium lactate in vacuum-packed meat products stored at temperatures from 1-7°C (Unda et al., 1991; Qvist et al., 1994). Weaver and Shelef (1993) found that 3% but not 2% (w/w) sodium lactate prevented growth of the pathogen in pork liver sausage at 5°C, whereas 2.5% (w/w) sodium lactate failed to prevent growth of the bacterium in turkey slurries stored at 4°C. Tan and Shelef (2002) stated that the microbial stability of ground pork stored at 2°C was enhanced by a combination of 2% sodium lactate and 1 to 2% NaCl.

3. MATERIALS AND METHODS

3. 1. Strains Used

Three strains of *B. cereus* were used in this study. Strains DSMZ 4312 (emetic) and DSMZ 4313 (diarrhoeal) were purchased from DSMZ – Braunschweig, Germany. Strain NRRL B-3711 was obtained from Agricultural Research Center of Midwest Area National Center for Agricultural Utilization Research, Illinois, USA.

3. 2. Media

Brain Heart Infusion Broth (BHI; Difco, Detr., MI) and Trypton Soy Agar (TSA; Oxoid, Hampshire) were used as the growth medium throughout the study.

NaCl (Merck, Darmstadt) was added to BHI to obtain final NaCl concentrations of 2.0 % (342 mM) and 3.5 % (600 mM). Since BHI contains 0.5 % (85 mM) NaCl, this concentration was selected as the base level for NaCl. Appropriate amounts of sodium lactate (50 % w/v, Merck, Darmstadt) was added to the experimental broth to obtain final concentrations of 0, 200 mM (1.7 %), 400 mM (3.5 %), 600 mM (5.0 %). After all, the pH of the media was adjusted using 4 N KOH (Merck, Darmstadt) or 4 N HCl (Merck, Darmstadt) solution to pH 4.8, 5.3, 5.8, 6.3, 6.8 and 7.3 and the media was sterilized at 121°C for 15 min. After sterilization the pH of the media was checked. Water activity were measured using an AquaLab Water Activity Meter (Decagon, Washington, USA).

3. 3. Determination of Minimum Growth Temperature and pH

Strains of *B. cereus* grown individually in BHI broth for 18 h at 30°C were inoculated into BHI broth and incubated at 4°C, 6°C, 8°C, 10°C and 30°C to determine the minimum growth temperature and also to determine whether the strains are mesophilic or psychrotrophic. Incubation was continued for 30 days to conclude that

growth was not observed. In the same way, the strains were inoculated into the growth media with pH levels of 4.8, 5.0, 5.3 and 5.5 and incubated at 30°C for 30 days to determine the minimum pH level for growth. Cultures were scored as no growth if the optical density failed to rise more than 0.1 OD unit above the value measured at inoculation.

3. 4. Preparation of Inocula and Growth Measurements

Strains of *B. cereus* were grown individually in BHI broth (Difco) for 18 h at 30°C and then inoculated into the experimental broths at equal concentrations to obtain a mixed culture with final concentration of approximately 10^6 cfu/ml.

Microbial growth was monitored by measuring the optical density (OD) (Milton Roy Spectronic 1201 Spectrophotometer) at 600 nm of the culture suspensions that were aseptically removed from the experimental flasks according to the procedure described by Baker and Griffiths (1993). Between 7 to 20 optical density measurements were used to construct each curve. Points at which no growth was observed in 30 days, were accepted as no growth conditions.

In order to convert OD values to CFU (colony forming unit), individual calibration curves were obtained for each strain (Figures 3. 1- 3. 3). For this, a concentrated culture was prepared for each strain in BHI at 30°C, and serial dilutions were made from stock culture solution. The OD values for the dilutions were measured with the spectrophotometer and also the plate counts were obtained for each. Calibration curves were drawn by plotting optical density (OD) at 600 nm versus viable number of bacteria (log cfu/ml) as described by Hudson (1994). An average of these curves was used for the viable count calculations of the mixed culture.

The initial optical density of the experimental samples containing equal concentrations of the three strains were approximately 0.04. Optical density is linearly correlated with viable plate count to the maximum optical density used (0.5 unit). Above this value the relationship became non-linear and hence cultures with optical densities above 0.5 unit were diluted in medium until they measured less than this value. Correction was then made for the amount by which the culture had been diluted.

3. 5. Experimental Design

Data were obtained using a fractional factorial design (Table A. 1) plus a full factorial experimental design (Table A. 2) and using combinations of factors that were randomly selected. The effects of temperature (8°C, 15°C, 26°C, 32°C), pH (5.3, 5.8, 6.3, 6.8, 7.3), NaCl concentration (0.5%, 2%, 3.5%) and sodium lactate concentration (0, 200 mM, 400 mM, 600 mM) were investigated simultaneously. Some of the conditions were replicated twice.

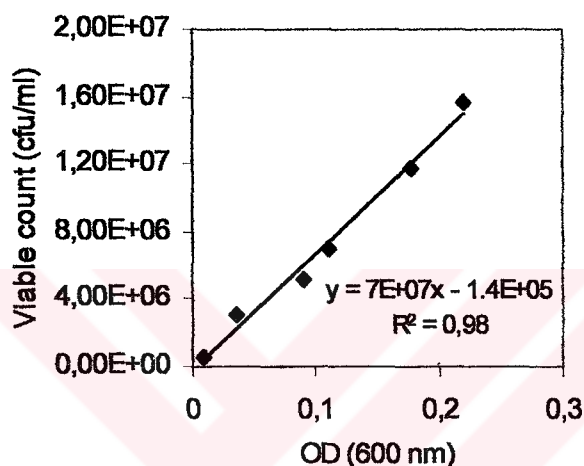


Figure 3. 1. Relationship between Optical density (OD) at 600 nm and viable cell numbers for *B. cereus* strain DSMZ 4313.

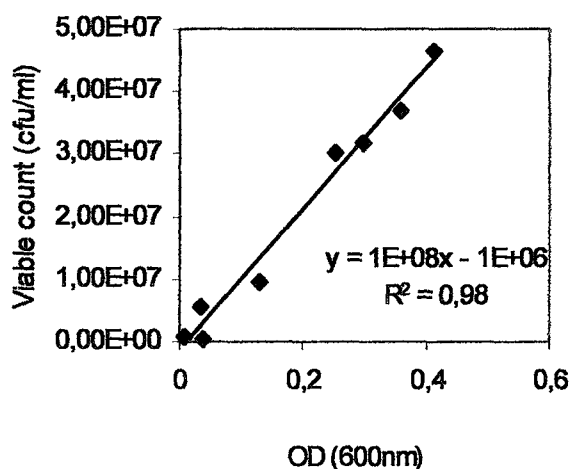


Figure 3. 2. Relationship between Optical density (OD) at 600 nm and viable cell numbers for *B. cereus* strain DSMZ 4312.

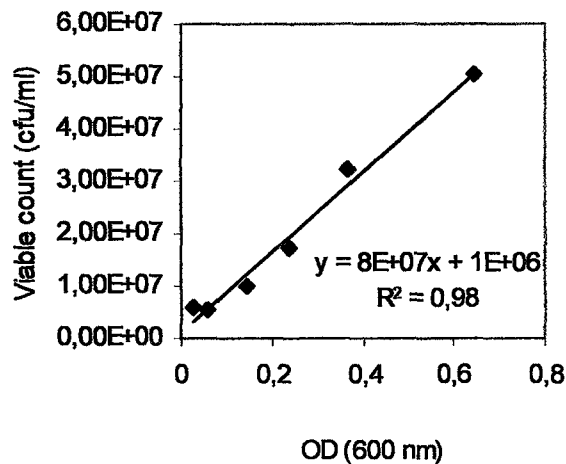


Figure 3. 3. Relationship between Optical density (OD) at 600 nm and viable cell numbers for *B. cereus* strain NRRL B-3711.

3. 6. Validation

Milk and meat broth were used for the validation studies. Four different meat broth samples were prepared with the addition of appropriate amounts of sodium lactate (0, 200, 400 mM) and sodium chloride (85 mM, 172 mM) to adjust the required concentrations. After sterilization of milk and meat broth samples, mixed *B. cereus* culture was inoculated at a level of 10^4 cfu/ml to the samples. They were transferred aseptically in 10 ml portions into test tubes. Milk samples were incubated at 12°C and 18°C, and meat broths were incubated at 18°C. Samples were taken to obtain plate counts. Sampling times were determined by taking into account the growth kinetic data obtained from the model derived in the first part of the study. Growth curves were constructed using the plate count data.

3. 7. Calculation of the Growth Parameters and Statistical Analysis

In order to obtain a reliable estimation of the parameters, all the growth curves were fitted to the Gompertz function using STATISTICA Non-Linear Regression procedure. Using Gompertz parameters (A, B, C and M), lag-time and growth rate were calculated for each condition.

Polynomial models describing the effects of temperature, pH, NaCl and sodium lactate concentration on the growth rate and lag time were obtained using Stepwise Regression procedure with STATISTICA. Using these equations three dimensional

response surface graphs were constructed for lag-time and growth rate versus the independent variables in STATISTICA.

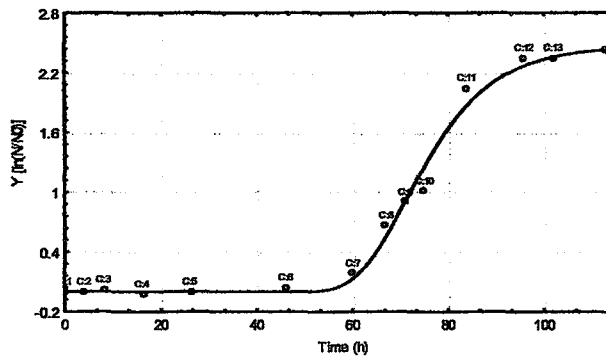


4. RESULTS AND DISCUSSION

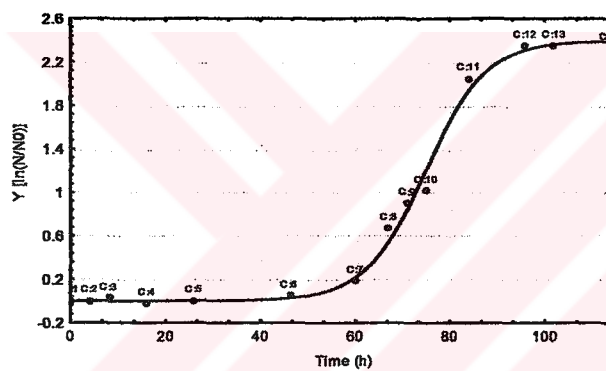
4. 1. Curve Fitting and Selection of the Primary Model

Excluding the replications, a total of 75 growth curves representing different combinations of temperature, pH, sodium chloride concentration and sodium lactate concentration were generated using the three parameter models Gompertz and logistic equations, and a four parameter model, that is the Stannard equation. The statistical data of these curves are given in Table 4.1. Between 10-20 observations were taken during the growth period for each treatment combination. Almost all the data fitted to the curves with R^2 's of greater than 0.95, examples of which are given in Fig. 4. 1. In order to select the best model among these three equations f-testing was applied. Assuming that the Stannard model exactly predicts the number of organisms, the RSS of the Stannard model was taken as an estimate of the measuring error, as explained by Zwietering et al. (1990). For the Gompertz model, the f-testing value was higher than the F table value in 12 of the 75 cases. This means that the Gompertz model was statistically accepted as a sufficient model in 63 (84 %) of the 75 cases. Similarly, for logistic model, this ratio was 61 of the 75 cases (81%), and thus both models seem to be sufficient to describe the data. But, in 52 of the 75 growth curves, the RSS of the Gompertz model was lower than that of the logistic equation. Moreover, even in the remaining 23 curves, the Gompertz model still fitted the data acceptably. Actually the Stannard equation gave a lower RSS value only in 24 out of 75 cases, and in the others the RSS values for Gompertz and Stannard equations were almost equal. As stated by Zwietering et al. (1990), if a three-parameter model is sufficient to describe the data, it is recommended over a four-parameter model, because it is simpler and the estimates of it have more degrees of freedom. Another important point is that, in three-parameter models all three parameters can be given a biological meaning. Therefore Gompertz model was selected to be used for the calculation of growth kinetic parameters to be used in further modelling studies, as also found by Zwietering et al. (1990) for many bacteria like *Staphylococcus aureus*, *Lactobacillus plantarum*, *Listeria monocytogenes*, *Salmonella heidelberg*, *Pseudomonas putida*.

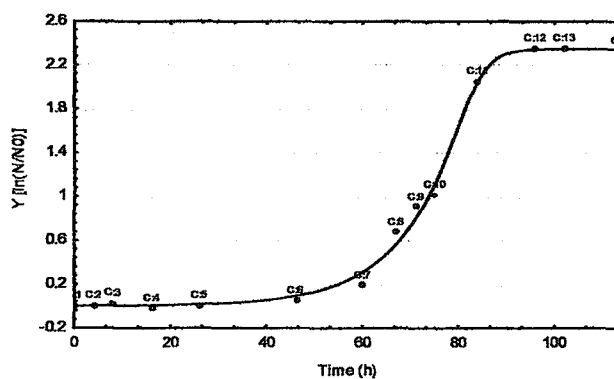
Including the replicates, a total of 95 growth curves (Appendix B) were generated using Gompertz equation (Equation 2. 3). Using equations 2. 3. a-c, the growth rate (log increase / h), the generation time (h) and the lag phase duration (h) were calculated from those fitted curves (Table 4. 2).



(a)



(b)



(c)

Figure 4. 1. Growth curves of *B. cereus* at 8°C, pH 6.8, and 85 mM sodium chloride fitted with; (a) Gompertz model, (b) logistic model, and (c) Stannard model .

4. 2. Modeling the Growth Kinetic Parameters

The general polynomial model to fit the data using 95 growth curves was in the below form:

$$\ln y = B_0 + B_1T + B_2PH + B_3NAL + B_4NACL + B_5T^2 + B_6PH^2 + B_7NAL^2 + B_8NACL^2 + B_9T*PH + B_{10}T*NAL + B_{11}T*NACL + B_{12}PH*NAL + B_{13}PH*NACL + B_{14}NAL*NACL \quad (4.1)$$

where y is the lag time or growth rate, B_i the parameter coefficients, T is temperature, PH is the pH level, NAL is the sodium lactate concentration in mM, $NACL$ is the sodium chloride concentration in mM.

The Arrhenius-Davey model given in Equation 2.10 was modified to include the sodium lactate concentration and sodium chloride concentration as follows:

$$\ln k = C_0 + C_1 / T + C_2 / T^2 + C_3PH + C_4PH^2 + C_5NAL + C_6NAL^2 + C_7NACL + C_8NACL^2 \quad (4.2)$$

where k is the growth rate or the reciprocal of lag time ($1/LAG$).

Detailed statistical analysis of the models used to describe the lag time and growth rate depending on the independent variables (temperature, pH, sodium lactate and sodium chloride concentrations) are given in Appendix C. As seen from the distribution of residual graphs and residual versus predicted value graphs (Figure C. 1 to C. 16), in order to obtain a residual distribution close to that of a normal distribution and to produce a more homogeneous error structure over the region of experimental design, it was better to use the natural logarithm of lag time and growth rate in modelling as stated by Gibson et al. (1988) and Baranyi et al. (1996).

The lack of fit analysis data for the polynomial models and the modified Arrhenius-Davey model for lag time and growth rate of *B. cereus* are given in Table 4. 3. The f values for all models were found to be higher than the table value of F , indicating a significant lack of fit for the models. However since the R^2 values for all the models were sufficiently high, meaning that much of the variation is already accounted for by the models, there is no way for improving the models (Box and Draper, 1987). Therefore polynomial models were chosen to be used in further discussion of the modelling study as the f values of them were much closer to the table F values, and they have higher R^2 values indicating that they described the variance of the data better.

Table 4. 1. Statistical-analytical data for 75 growth curves of *B. cereus*.

Curve no	RSS _{Gompertz}	RSS _{Logistic}	RSS _{Standard}	df ₁	df ₂	$F_{(\alpha=0.05, df_1, df_2 - df_1)}$	$\hat{f}_{Gompertz}$	$\hat{f}_{Logistic}$
1	0,13	0,08	0,05	10	11	4,96	15,16	5,49
2	0,02	0,06	0,02	10	11	4,96	0,08	16,67
3	0,02	0,06	0,02	13	14	4,67	0,07	25,32
4	0,54	0,66	0,54	12	13	4,75	-0,01	2,77
5	0,28	0,19	0,12	5	6	6,61	6,75	3,08
6	0,06	0,05	0,05	5	6	6,61	0,50	0,40
7	0,15	0,10	0,06	7	8	5,59	11,18	5,40
8	0,08	0,07	0,07	11	12	4,84	1,64	0,82
9	0,27	0,36	0,27	6	7	5,99	0,00	1,91
10	0,69	0,67	0,67	10	11	4,96	0,38	0,00
11	0,35	0,41	0,35	14	15	4,60	0,01	2,44
12	0,12	0,10	0,10	8	9	5,32	1,74	0,11
13	0,03	0,03	0,03	10	11	4,96	1,63	2,02
14	0,37	0,45	0,38	9	10	5,12	0,00	1,68
15	0,03	0,01	0,01	5	6	6,61	15,83	2,50
16	0,18	0,30	0,18	10	11	4,96	0,00	6,82
17	0,25	0,29	0,25	14	15	4,60	0,00	2,08
18	0,16	0,09	0,03	7	8	5,59	38,10	18,18
19	0,31	0,37	0,31	9	10	5,12	0,00	1,77
20	0,95	0,69	0,26	10	11	4,96	26,25	16,32
21	0,75	0,74	0,66	7	8	5,59	0,95	0,93
22	0,60	0,41	0,16	12	13	4,75	32,44	18,44
23	0,29	0,29	0,29	12	13	4,75	0,02	0,35
24	0,18	0,29	0,18	12	13	4,75	0,00	7,74
25	0,23	0,31	0,23	12	13	4,75	0,00	4,31
26	0,37	0,47	0,37	10	11	4,96	0,00	2,60
27	1,51	1,89	1,51	9	10	5,12	0,00	2,24
28	0,26	0,13	0,26	8	9	5,32	0,00	-4,14
29	0,84	1,24	0,84	6	7	5,99	0,00	2,86
30	1,57	1,91	1,57	13	14	4,67	0,00	2,79
31	0,30	0,52	0,30	6	7	5,99	0,00	4,58
32	1,09	1,17	1,09	8	9	5,32	0,00	0,59
33	0,22	0,34	0,22	10	11	4,96	0,00	5,39
34	0,71	0,92	0,71	13	14	4,67	0,00	3,86
35	0,20	0,20	0,20	10	11	4,96	0,00	0,15
36	0,58	0,64	0,58	11	12	4,84	0,00	1,10
37	1,70	2,25	1,70	6	7	5,99	0,00	1,96
38	0,78	1,01	0,78	5	6	6,61	0,00	1,52
39	1,41	1,81	1,41	8	9	5,32	-0,01	2,24
40	0,57	0,63	0,57	8	9	5,32	0,00	0,79
41	0,64	0,65	0,64	10	11	4,96	0,00	0,22
42	0,51	0,44	0,44	6	7	5,99	0,87	0,01
43	2,17	1,71	0,94	6	7	5,99	7,95	4,98

Table 4. 1. Statistical-analytical data for 75 growth curves of *B. cereus* (cont.).

44	0,41	0,47	0,41	7,00	8,00	5,59	0,00	1,06
45	0,31	0,40	0,31	8,00	9,00	5,32	0,00	2,49
46	1,95	2,25	1,95	9,00	10,00	5,12	0,00	1,38
47	0,44	0,50	0,44	9,00	10,00	5,12	0,00	1,30
48	0,30	0,30	0,33	9,00	10,00	5,12	-0,86	-0,84
49	0,33	0,41	0,33	8,00	9,00	5,32	0,00	1,93
50	0,24	0,33	0,24	9,00	10,00	5,12	0,00	3,57
51	0,64	0,42	0,24	6,00	7,00	5,99	9,98	4,45
52	0,14	0,07	0,06	8,00	9,00	5,32	9,84	0,92
53	1,78	1,91	1,78	9,00	10,00	5,12	0,00	0,64
54	0,22	0,42	0,22	7,00	8,00	5,59	0,00	6,46
55	0,61	0,53	0,29	8,00	9,00	5,32	8,66	6,46
56	0,09	0,15	0,09	6,00	7,00	5,99	0,21	4,67
57	0,41	0,55	0,41	5,00	6,00	6,61	0,00	1,67
58	0,07	0,22	0,07	9,00	10,00	5,12	0,00	17,88
59	0,96	0,94	0,95	7,00	8,00	5,59	0,10	-0,03
60	0,82	0,91	0,82	9,00	10,00	5,12	0,00	0,98
61	0,31	0,31	0,30	11,00	12,00	4,84	0,25	0,33
62	0,92	1,45	0,92	6,00	7,00	5,99	0,00	3,45
63	0,40	0,60	0,40	7,00	8,00	5,59	0,00	3,34
64	0,61	0,89	0,61	9,00	10,00	5,12	0,00	4,15
65	0,09	0,12	0,09	6,00	7,00	5,99	0,01	1,62
66	1,11	1,15	1,11	9,00	10,00	5,12	0,00	0,28
67	0,18	0,26	0,18	6,00	7,00	5,99	0,00	2,88
68	0,06	0,22	0,06	6,00	7,00	5,99	-0,01	15,98
69	0,87	1,90	1,72	7,00	8,00	5,59	-3,44	0,73
70	0,24	0,42	0,24	9,00	10,00	5,12	0,00	6,51
71	0,15	0,06	0,15	5,00	6,00	6,61	0,01	-2,96
72	0,44	0,39	0,26	8,00	9,00	5,32	5,88	4,22
73	0,08	0,10	0,08	9,00	10,00	5,12	0,00	1,79
74	0,27	0,21	0,20	9,00	10,00	5,12	2,69	0,35
75	0,16	0,11	0,11	9,00	10,00	5,12	4,25	0,08

The polynomial models developed for the natural logarithm of lag time and growth rate were reduced by backward stepwise regression analysis to include only the coefficients significant at a level of $p < 0.05$ (Table C. 3 and C. 7). The final equations together with the root mean square error (RMSE) and coefficient of determination (R^2) values are given in Table 4. 4.

The RMSE provides a measure to judge the goodness of fit of the polynomial model. The lower the value of RMSE, the better is the fit (Sutherland et al., 1994).

Table 4. 2. Growth rate (GR), lag time (LAG) and generation time (GT) derived from calculated Gompertz curve parameters for growth of *B. cereus* in BHI broth.

Sample no	T (°C)	pH	NAL (mM)	NACL (mM)	LAG (h)	GR (log increase / h)	GT (h)
1.1	8	6,8	0	85	61,0	0,04	7,7
1.2	8	6,8	0	85	60,4	0,04	8,1
2.1	8	7,3	0	85	75,7	0,03	9,8
2.2	8	7,3	0	85	74,3	0,03	9,4
3.1	8	7,3	200	85	104,7	0,02	13,2
3.2	8	7,3	200	85	102,6	0,03	11,5
4.1	8	7,3	0	342	95,5	0,02	15,2
4.2	8	7,3	0	342	102,2	0,02	13,5
5.1	15	5,3	0	85	24,1	0,03	8,9
5.2	15	5,3	0	85	27,4	0,04	8,3
6.1	15	5,8	0	85	25,7	0,04	7,7
6.2	15	5,8	0	85	23,0	0,04	7,1
7.1	15	5,8	200	85	41,8	0,03	11,1
7.2	15	5,8	200	85	47,3	0,03	9,6
8.1	15	5,8	200	600	78,5	0,02	14,7
8.2	15	5,8	200	600	81,5	0,03	12,3
9.1	15	6,3	0	85	15,1	0,11	2,8
9.2	15	6,3	0	85	14,4	0,10	3,0
10.1	15	6,3	200	85	29,5	0,06	4,9
10.2	15	6,3	200	85	27,1	0,06	5,3
11	15	6,3	400	85	85,5	0,03	11,1
12	15	6,3	0	600	38,9	0,03	9,1
13	15	6,3	200	600	79,0	0,02	14,9
14	15	6,3	200	342	63,8	0,03	12,0
15	15	6,8	0	85	15,9	0,14	2,1
16	15	6,8	200	85	27,6	0,05	6,0
17	15	6,8	400	85	85,4	0,03	10,3
18	15	6,8	200	342	49,5	0,03	9,2
19	15	6,8	200	600	76,3	0,03	11,8
20	15	7,3	0	85	21,4	0,12	2,5
21	15	7,3	200	85	22,2	0,08	3,7
22.1	15	7,3	400	85	35,9	0,04	8,4
22.2	15	7,3	400	85	36,1	0,04	7,9
23	15	7,3	600	85	67,0	0,03	10,5
24	15	7,3	0	600	39,3	0,07	4,3
25	15	7,3	200	600	62,7	0,04	8,3
26	15	7,3	200	342	49,0	0,05	6,4
27	26	5,3	0	85	2,5	0,31	1,0
28	26	5,3	0	600	19,0	0,24	1,3
29	26	5,8	0	85	1,5	0,39	0,8
30	26	5,8	200	85	8,8	0,06	5,5
31.1	26	6,3	0	85	0,9	0,44	0,7
31.2	26	6,3	0	85	1,2	0,42	0,7

Table 4. 2. Growth rate (GR), lag time (LAG) and generation time (GT) derived from calculated Gompertz curve parameters for growth of *B. cereus* in BHI broth (cont.).

32.1	26	6,3	200	85	2,8	0,18	1,7
32.2	26	6,3	200	85	2,9	0,20	1,5
33.1	26	6,3	400	85	38,0	0,04	8,7
33.2	26	6,3	400	85	33,5	0,03	9,8
34.1	26	6,3	0	600	7,7	0,28	1,1
34.2	26	6,3	0	600	7,2	0,27	1,1
35	26	6,3	200	600	46,8	0,06	5,4
36	26	6,3	200	342	21,6	0,06	4,7
37	26	6,8	0	85	1,1	0,54	0,6
38	26	6,8	200	85	3,2	0,20	1,5
39	26	6,8	400	85	18,1	0,04	7,7
40	26	6,8	200	342	21,3	0,06	5,4
41	26	6,8	200	600	44,7	0,05	6,1
42	26	7,3	0	85	1,1	0,49	0,6
43	26	7,3	200	85	1,8	0,31	1,0
44	26	7,3	400	85	11,8	0,06	4,9
45	26	7,3	600	85	40,1	0,04	8,6
46	26	7,3	0	600	8,0	0,13	2,3
47	26	7,3	200	600	20,7	0,05	5,6
48.1	26	7,3	400	600	40,9	0,03	9,3
48.2	26	7,3	400	600	42,7	0,03	9,5
49	26	7,3	200	342	14,5	0,06	4,9
50	26	7,3	400	342	33,7	0,04	8,7
51.1	32	5,3	0	85	1,5	0,36	0,8
51.2	32	5,3	0	85	2,1	0,39	0,8
52	32	5,3	0	600	18,4	0,23	1,3
53	32	5,8	0	85	1,0	0,41	0,7
54	32	5,8	200	85	7,0	0,20	1,5
55	32	5,8	200	342	25,6	0,08	3,8
56	32	6,3	0	85	0,8	0,49	0,6
57	32	6,3	200	85	2,6	0,21	1,4
58	32	6,3	400	85	28,2	0,04	7,0
59	32	6,3	0	600	8,4	0,24	1,3
60	32	6,3	200	600	33,8	0,05	5,9
61	32	6,3	200	342	15,0	0,08	3,9
62	32	6,8	0	85	0,7	0,95	0,3
63.1	32	6,8	200	85	1,6	0,30	1,0
63.2	32	6,8	200	85	4,4	0,34	0,9
64	32	6,8	400	85	12,7	0,05	5,7
65	32	6,8	200	342	19,6	0,08	4,0
66.1	32	6,8	200	600	33,7	0,06	4,9
66.2	32	6,8	200	600	31,5	0,06	5,2
67	32	7,3	0	85	1,0	0,63	0,5
68	32	7,3	200	85	1,2	0,34	0,9
69	32	7,3	400	85	6,7	0,06	4,9

Table 4. 2. Growth rate (GR), lag time (LAG) and generation time (GT) derived from calculated Gompertz curve parameters for growth of *B. cereus* in BHI broth (cont.).

70	32	7,3	600	85	26,8	0,04	7,9
71.1	32	7,3	0	600	6,3	0,17	1,8
71.2	32	7,3	0	600	5,6	0,15	1,9
72	32	7,3	200	600	27,7	0,07	4,2
73	32	7,3	400	600	37,5	0,03	9,1
74	32	7,3	200	342	19,3	0,06	4,7
75	32	7,3	400	342	35,1	0,04	8,4

Following equation 17.c, it was found that, between the experimental region, the error of the prediction of lag time was about 38 % and that of growth rate was about 31 % of the prediction itself. Moreover, the percentage of variance accounted for lag time and growth rate was 95 % and 94 %, respectively. As shown from the scatter plots of observed versus predicted lag time in Figure 4. 2 and observed versus predicted growth rate in Figure 4. 3, the fitted values compare well with those measured, indicating that the models provide a good description for the data used to generate them. Fernandez et al. (1997) and Eifert et al. (1996) also used scatter plots of observed versus predicted data to assess the success of the model in predicting the data used to generate it.

While the models were produced using growth rates, comparisons were made using the generation time, since this value is more uniformly used in the literature and avoids confusion between different growth rate units.

Table 4. 3. Analysis of lack of fit.

Model	RSS ₁	df ₁	RSS ₂	df ₂	f	$F_{(\alpha=0.05, df_1, df_2 - df_1)}$	% R ²
Ln (LAG) (polynomial)	0.677	20	8.253	80	3.73	1.95	95.5
Ln (1/LAG) (Arrhenius-Davey)	0.976	20	20.352	86	6.01	1.95	88.9
Ln (GR) (polynomial)	0.092	20	5.746	80	20.56	1.95	94.0
Ln (GR) (Arrhenius-Davey)	0.092	20	14.049	86	45.97	1.95	85.4

4. 3. Comparison of the InLAG and InGR Models with USDA-PMP (PMP)

In Table 4. 5, the lag times and generation times predicted by the model and the mean predictions of the PMP together with their confidence limits are given. Almost in all cases the lag times and generation times predicted by the models were

Table 4. 4. Reduced polynomial models for lag time (LAG) and growth rate (GR).

Model	RMSE	% R ²
$\ln \text{LAG} = 21.462 - 0.388*T - 4.222*PH + 0.0143*NAL + 0.003*NACL + 0.004*T^2 + 0.303*PH^2 - 0.0000065*NACL^2 + 0.000189*T*NAL + 0.000175*T*NACL - 0.00195*PH*NAL$	0.32	95.2
$\ln \text{GR} = -20.251 + 0.411*T + 3.743*PH - 0.0096*NAL - 0.0034*T^2 - 0.2220*PH^2 + 0.00000603*NACL^2 - 0.0203*T*PH - 0.00015*T*NAL - 0.000058*T*NACL + 0.0013*PH*NAL - 0.0007*PH*NACL$	0.27	93.9

in between the upper and lower confidence limits of the PMP predictions. Moreover, the comparison of the predictions of the derived models for lag time and generation time with the PMP (Figure 4. 4 and 4. 5) showed that the model predictions compare well with the PMP predictions both for lag time and generation time in most cases.

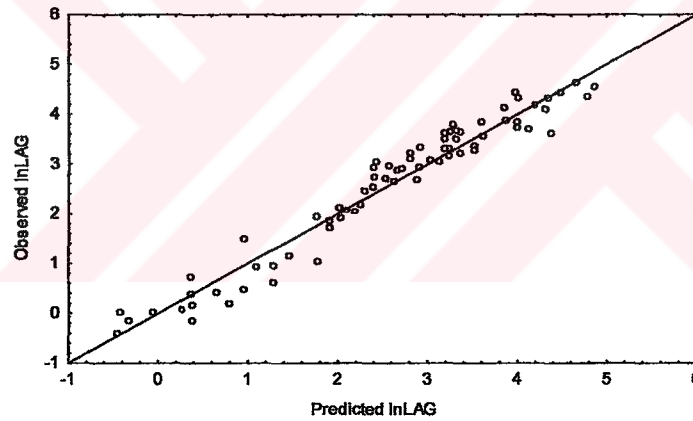


Figure 4. 2. Observed versus predicted ln lag time.

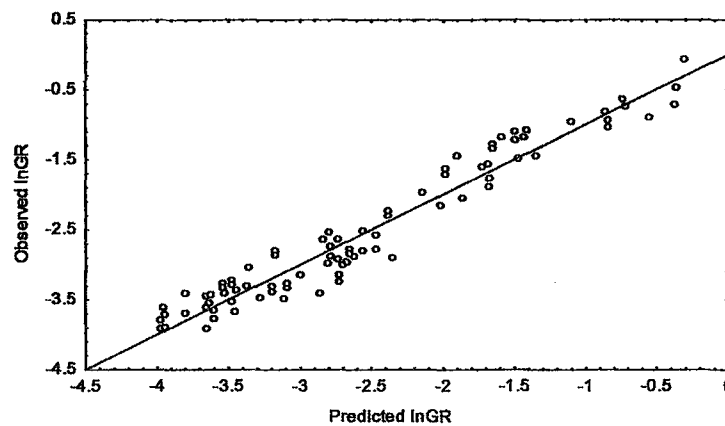


Figure 4. 3. Observed versus predicted ln growth rate.

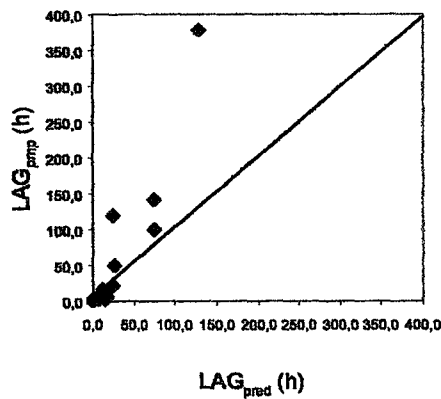


Figure 4. 4. Comparison of lag times predicted by the model and lag times calculated by PMP.

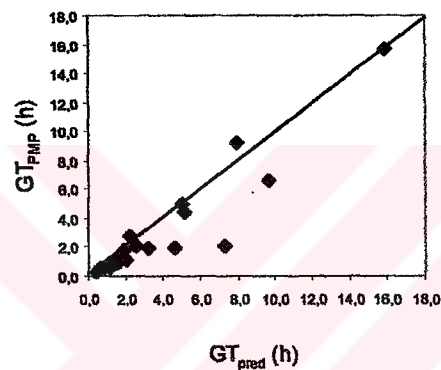


Figure 4. 5. Comparison of generation times predicted by the model and generation times calculated by PMP.

4. 4. Validation of the Models

The data obtained from the validation studies done in milk and meat broth are given in Table 4. 6. The respective growth curves are given in Appendix D. Scatter plots (Figure 4. 6 and 4. 7) were used for the comparison of the predicted and observed values, as done by Sutherland et al. (1994), McClure et al. (1993), Fernandez et al. (1997) and Sutherland et al. (1996). The lag time was predicted longer than the observed value only in one case, and the rest of the lag time predictions fell in the fail-safe region (Figure 4. 6). In only two of the cases, the model predicted the growth rate higher than the observed growth rate. However, the predictions were still satisfactory since they were very close to the observed values (Figure 4. 7).

Table 4. 5. Comparison of model predictions with the predictions of PMP for the lag time and the generation time of *B. cereus* in liquid media.

T (°C)	pH	NAL (mM)	NACL (mM)	LAG _{pred} (h)	GT _{pred} (h)	LAG _{PMP} (h)			GT _{PMP} (h)		
						LCL*	UCL*	Mean	LCL	UCL	Mean
8	6,8	0	85	61,0	7,7	65,5	149,8	99,1	4,3	10,2	6,6
8	6,8	0	85	60,4	8,1	65,5	149,8	99,1	4,3	10,2	6,6
8	7,3	0	85	75,7	9,8	74,2	269,4	141,4	4,7	18,1	9,2
8	7,3	0	85	74,3	9,4	74,2	269,4	141,4	4,7	18,1	9,2
8	7,3	0	342	95,5	15,2	182,2	779,4	376,9	7,59	32,47	15,7
8	7,3	0	342	102,2	13,5	182,2	779,4	376,9	7,59	32,47	15,7
15	5,3	0	85	24,1	8,9	13,4	37,2	22,3	1,2	3,6	2,1
15	5,3	0	85	27,4	8,3	13,4	37,2	22,3	1,2	3,6	2,1
15	5,8	0	85	25,7	7,7	10	20,2	14,2	1,3	2,7	1,9
15	5,8	0	85	23,0	7,1	10	20,2	14,2	1,3	2,7	1,9
15	6,3	0	85	15,1	2,8	8,7	15,6	11,6	1,4	2,6	1,9
15	6,3	0	85	14,4	3,0	8,7	15,6	11,6	1,4	2,6	1,9
15	6,3	0	600	38,9	9,1	31,3	80,7	50,3	2,7	7,2	4,4
15	6,8	0	85	15,9	2,1	8,9	17,2	12,3	1,5	3	2,1
15	7,3	0	85	21,4	2,5	10	28,5	16,9	1,6	4,7	2,8
15	7,3	0	600	39,3	4,3	51,7	274,3	119,1	2,15	11,43	4,96
26	5,3	0	85	2,5	1,0	2,5	5	3,6	0,5	1,1	0,8
26	5,3	0	600	19,0	1,3	3,6	10,7	6,2	0,6	1,9	1,1
26	5,8	0	85	1,5	0,8	1,5	3	2,1	0,4	0,8	0,6
26	6,3	0	85	0,9	0,7	1,1	2,3	1,6	0,4	0,8	0,5
26	6,3	0	85	1,2	0,7	1,1	2,3	1,6	0,4	0,8	0,5
26	6,3	0	600	7,72	1,1	3	7,1	4,6	0,7	1,6	1,1
26	6,3	0	600	7,25	1,1	3	7,1	4,6	0,7	1,6	1,1
26	6,8	0	85	1,1	0,6	1,1	2,3	1,6	0,4	0,8	0,5
26	7,3	0	85	1,1	0,6	1,3	3,2	2,1	0,4	1	0,6
26	7,3	0	600	8,0	2,3	4,4	21,3	9,6	0,8	3,9	1,8
32	5,3	0	85	1,5	0,8	1,7	3,7	2,5	0,3	0,8	0,5
32	5,3	0	85	2,1	0,8	1,7	3,7	2,5	0,3	0,8	0,5
32	5,3	0	600	18,4	1,3	2	5,8	3,4	0,4	1,2	0,7
32	5,8	0	85	1,0	0,7	1	2,1	1,4	0,3	0,6	0,4
32	6,3	0	85	0,8	0,6	0,7	1,5	1,1	0,2	0,5	0,3
32	6,3	0	600	8,4	1,3	1,5	3,8	2,4	0,4	1	0,6
32	6,8	0	85	0,7	0,3	0,7	1,4	1	0,2	0,4	0,3
32	7,3	0	85	1,0	0,5	0,9	1,8	1,3	0,2	0,5	0,3
32	7,3	0	600	6,33	1,8	2	11	4,6	0,4	2,1	0,9
32	7,3	0	600	5,61	1,9	2	11	4,6	0,4	2,1	0,9

*UCL and LCL are the 95 % confidence intervals of the estimated means.

Table 4. 6. Comparison of observed and predicted lag time and growth rate of *B. cereus* in milk and meat broth.

Sample name	T (°C)	pH	NAL (mM)	NaCl (mM)	LAG _{obs} (h)	GR _{obs} (log increase/h)	LAG _{pred} (h)	GR _{pred} (log increase/h)
Milk	12	6.68	0	85	19.7	0.04	24.0	0.07
Milk	18	6.68	0	85	12.3	0.20	3.7	0.24
Meat broth	18	6.34	0	85	12.1	0.19	6.1	0.16
Meat broth	18	6.34	200	85	23.8	0.07	16.7	0.07
Meat broth	18	6.34	400	85	74.4	0.04	45.8	0.03
Meat broth	18	6.34	400	172	85.7	0.04	69.6	0.02

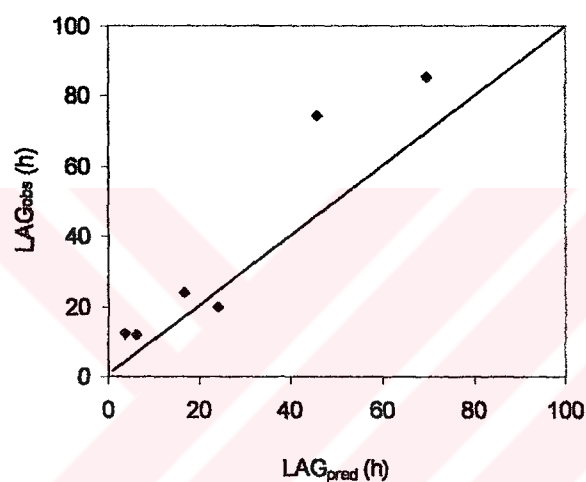


Figure 4. 6. Comparison of predicted and observed lag times of *B. cereus* in milk and meat meal.

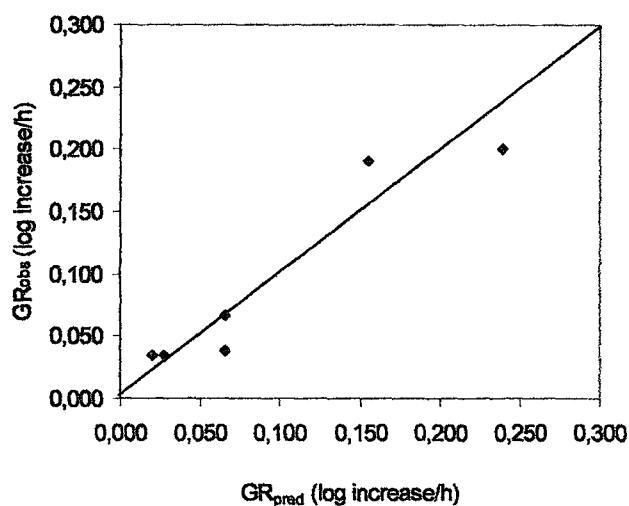


Figure 4. 7. Comparison of predicted and observed growth rates of *B. cereus* in milk and meat meal.

Table 4. 7 shows some examples of published data in various foods and growth media compared with the predicted generation times by our model (InGR) and also by PMP model. From these data, the % RE and the MARE of the predictions were calculated using equation 13 and 14 (Table 4. 8). For both of the models, the MRE showed that the distribution of the RE was slightly biased towards the safe side as shown in Figure 4. 8 and 4. 9. Only a few of the data points fell in the "unsafe side", that is the side in which the generation time of the *B. cereus* was predicted longer than the observed generation time (Figure 4. 8). Most of the points fell below the line of 100% correlation ($y=x$) indicating that the models 'fail-safe'. The MARE of our model was found to be lower than that of PMP, indicating that our model predicted the generation time of *B. cereus* more accurately. Moreover, as done by comparing the MARE of generation time models for various bacteria showed that the MARE of our model was lower than most of the published models, indicating that the current model could be safely used for generation time predictions of *B. cereus* within the range of factors modeled (Table 4. 9).

As shown in Figure 4. 8, some of the points fell very far from the line of unity having very high relative errors, even higher than 50 % (Figure 4. 9). Actually this was not surprising, because various strains of the same species may have very different growth behavior, with generation times or lag phase durations differing 2-3 times depending on the strain. For example, Wong and Chen (1988) reported 0.3 h and 0.6 h of generation times at the same conditions in milk for emetic and diarrhoeal strains of *B. cereus*, respectively. Similarly, Sooltan et al. (1987) reported 4.1 h and 1.4 h of generation times under the same conditions in cooked poultry breast for different strains of *B. cereus*. Another reason for high percentage RE may be that the growth media used in the model development may not always be a good model system for the food of concern, and thus the predictions could be very high or very low from the observed parameter. If the food is a better medium than the growth medium used for the growth of the bacterium, than it is normal to observe lower lag phase durations or higher growth rates than that of predicted. Shaking during incubation, the type of acidulant used, physiological state of the bacteria used for inoculation, prior temperature history of the cells are some other factors that could cause significant differences in the growth kinetics of bacteria (Walker et al., 1990; Buchanan and Klawitter, 1991). Therefore differences in these factors may also give rise to very high % RE for the model predictions compared to observed data in foods.

Table 4. 7. Comparison of observed and predicted generation times for *B. cereus* in foods and different growth media, without sodium lactate.

T (°C)	pH	NACL (mM)	Substrate	No of strain s	GT _{obs}	GT _{pred}	GT _{PMP}	Reference
30	6,8	85	Non-fat milk medium	1	0,3	0,5	0,4	Wong and Chen, 1988
30	6,8	85	Non-fat milk medium	1	0,6	0,5	0,4	Wong and Chen, 1989
22	6,5	85	Cooked poultry leg	1	1,1	0,9	0,8	Sooltan et al., 1987
22	6,5	85	Cooked poultry leg	1	1,3	0,9	0,8	Sooltan et al., 1987
15	6	85	Cooked poultry breast	1	3,2	3,0	1,8	Sooltan et al., 1987
15	6	85	Cooked poultry breast	1	4,1	3,0	1,8	Sooltan et al., 1987
22	6	85	Cooked poultry breast	1	1,4	1,1	0,8	Sooltan et al., 1987
15	6,5	85	Cooked poultry leg	1	2,6	2,0	1,9	Sooltan et al., 1987
15	6,5	85	Cooked poultry leg	1	3,7	2,0	1,9	Sooltan et al., 1987
15	6	297,04	Liver sausage	1	8,3	7,0	1,8	Asplund et al., 1988
20	6,4	85	Reconst. dried milk	>1	2,2	1,1	1,0	Rodriquez and Barrett, 1986
26	6,5	85	Infant formula	1	0,9	0,7	0,5	Harmon and Kautter, 1991
30	6,7	85	Pasteurised milk	1	0,5	0,5	0,4	Wong et al., 1988
15	7	85	Cooked rice	1	3,0	1,5	2,3	Johnson et al., 1983
15	7	85	Cooked rice	1	4,3	1,5	2,3	Johnson et al., 1983
30	7	85	Cooked rice	1	0,4	0,5	0,4	Gilbert and Stringer, 1974
30	7	85	Cooked rice	1	0,5	0,5	0,4	Gilbert and Stringer, 1974
22	6,1	85	Pinto beans	1	0,8	1,0	0,8	Nester and Woodburn, 1982
22	6,1	85	Pinto beans	1	1,2	1,0	0,8	Nester and Woodburn, 1982
22	7	85	Red lentils (cooked)	1	1,7	0,8	0,9	Blakey and Priest, 1980
22	7	85	Kidney beans (cooked)	1	1,7	0,8	0,9	Blakey and Priest, 1980
15	7	85	Rice+10% beef extract	4	3,3	1,5	2,3	Johnson et al., 1983
25	7	85	Rice+10% beef extract	4	0,7	0,7	0,6	Johnson et al., 1983
30	6,8	85	Milk	1	0,4	0,5	0,4	Wong et al., 1988
15	7	85	TSB	4	2,1	1,5	2,3	Johnson et al., 1983
15	7	85	TSB	4	3,2	1,5	2,3	Johnson et al., 1983
25	7	85	TSB	4	0,7	0,7	0,6	Johnson et al., 1983
25	7	85	TSB	4	1,0	0,7	0,6	Johnson et al., 1983
30	7	85	TSB	4	0,5	0,5	0,4	Johnson et al., 1983
30	7	85	TSB	4	0,8	0,5	0,4	Johnson et al., 1983
20	6,4	85	Reconst. dried milk	>1	1,3	1,1	1,0	Rodriguez and Barret, 1986
30	6,4	85	Reconst. dried milk	>1	0,6	0,6	0,4	Rodriguez and Barret, 1986
30	5	85	BHI	4	0,8	0,8	0,7	Garcia-Arribas and Kramer, 1990
30	5	85	BHI	4	2,1	0,8	0,7	Garcia-Arribas and Kramer, 1990
30	6,8	85	BHI+1% starch	4	0,6	0,5	0,4	Garcia-Arribas and Kramer, 1990
30	6,8	85	BHI+1% starch	4	0,6	0,5	0,4	Garcia-Arribas and Kramer, 1990

GT_{obs}; observed generation time, GT_{pred}; predicted generation time, GT_{PMP}; generation time predicted by USDA Pathogen Modelling Program, Reconst.; Reconstituted.

It is desirable that if a model errs then it errs on the safe side, that is underestimating the lag phase duration or generation times and overestimation of the growth rate as stated by Hudson (1994). In this point of view the model for

growth rate, which was used to calculate generation times, provided reasonable and safer estimates. However, such an analysis for lag phase duration model could not be done, since there was not enough published data for the lag phase duration of *B. cereus* in foods.

Table 4. 8. Comparison of the accuracy and bias of GT_{pred} and GT_{PMP} .

Model	MARE	MRE
GT_{pred}	25.6	-16.7
GT_{PMP}	32.8	-33.3

Table 4. 9. Mean absolute relative error (MARE) of some published models.

Genus	Cases	MARE	Reference
<i>Salmonella</i>	20	18.5	Gibson et al. (1988)
<i>Escherichia</i>	58	25.7	Buchanan et al. (1993)
<i>Staphylococcus</i>	74	30.4	Buchanan et al. (1993)
<i>Aeromonas</i>	61	72.0	Palumbo et al. (1992)
<i>Shigella</i>	31	20.8	Zaika et al. (1992)
<i>Aeromonas</i>	54	52.3	Palumbo et al. (1991)
<i>Yersinia</i>	30	27.4	Bhaduri et al. (1994)

4. 5. Effect of Temperature on Lag Phase Duration (LAG) and Growth Rate (GR) of *B. cereus*

The minimum growth temperature of *B. cereus* was determined by incubating at 4°C, 6°C, 8°C, 10°C, and 30°C. It was concluded that all the three strains were mesophilic, since all of them grew very rapidly at 30°C, slowly at 8°C, but did not grow below this point. Since the lowest temperature at which growth was observed was 8°C, it was taken as the minimum level for temperature. Similarly, Benedict et al. (1993) reported 8°C as the minimum and 37°C as the optimum temperature for the growth of *B. cereus*.

At 8°C, the generation time was in average 7.9 h at 6.8 pH. Similarly, for the *B. cereus* strains that grew in 7°C, Dufrenne et al. (1994) reported generation times between 8-15 h in BHI broth.

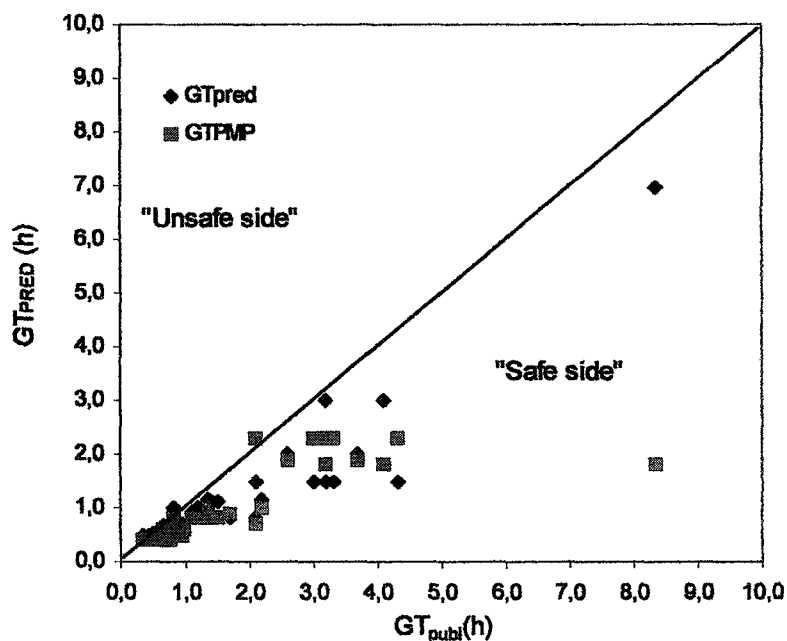


Figure 4. 8. Comparison of published generation times for *B. cereus* with the predicted generation times by the model and by PMP.

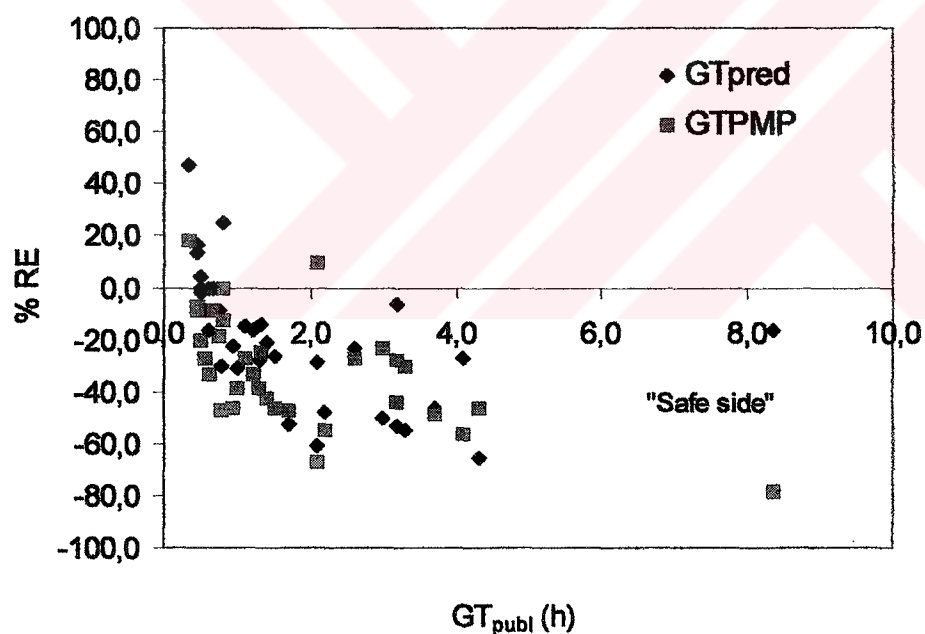


Figure 4. 9. Relative error (% RE) of generation time (GT) predictions from our model and PMP.

The fitted polynomial models for $\ln GR$ and $\ln LAG$ (Table 4. 4) showed that the temperature has a significant ($p \leq 0.05$) effect both on growth rate and lag phase duration in the studied experimental range. The growth rate was significantly affected by temperature, being maximal at 32°C and lowest at 8°C as expected. Actually, at 8°C, growth was hardly observed even at conditions of optimum or near

optimum pH levels, that is pH of 6.8-7.3 (Benedict et al., 1993). Even at 342 mM sodium chloride or 200 mM sodium lactate concentrations, the lag phase duration of the organism prolonged to about 100 hrs at 8°C. Choma et al. (2000) stated that *B. cereus* is not well adapted to cold and that any increase in storage temperature above 13°C would permit a dramatic increase in growth rate. This behavior is well demonstrated in Figure 4. 10 and 4. 11, in which examples of growth curves at different temperatures are shown. At 32°C which is in the reported optimum growth temperature range of *B. cereus* (ICMSF, 1996) and 26°C which is near the optimum range, the growth curves were very similar. However, at 15°C and 8°C the growth curves were easily separated with significantly longer lag phase durations and lower slope of the exponential phase line, indicating lower growth rates. At 8°C, the decrease in growth rate and increase in lag phase duration was more pronounced, since 8°C is at the limits of the minimum temperature for growth of *B. cereus*.

In Figure 4. 12 and 4. 13, the effect of temperature and sodium chloride, respectively on the lag phase duration and growth rate of *B. cereus* was demonstrated. The In LAG and In GR models (Table 4. 4) showed that the interaction between temperature and sodium chloride is significant at a level of $p < 0.05$. This was in agreement with the results of Baker and Griffith (1993). At higher temperatures, the rate of decrease in In LAG with decreasing sodium chloride concentrations was higher than that of at lower temperatures (Figure 4. 12). At lower temperatures, the In LAG first stayed constant up to sodium chloride concentrations of about 400 mM and then decreased only slightly with decreasing temperature. Similarly, the rate of change in In LAG with respect to temperature was also different at higher sodium chloride levels compared to at lower sodium chloride levels. At lower sodium chloride levels, the decrease in In LAG with respect to temperature was linear, whereas at higher sodium chloride levels the relation became parabolic. For growth rate (Figure 4. 13), at low sodium chloride concentrations, the increase in growth rate was higher with increasing temperature than that of at high sodium chloride concentrations as also observed by Baranyi et al. (1996) for *Salmonella*.

As also found by Baker and Griffith (1993), it was determined that the interaction of temperature and pH for In GR was significant at $p \leq 0.05$ (Table 4. 4). The decrease in temperature was more effective at low pH levels compared with at high pH levels (Figure 4. 14). Unlike for growth rate, for lag phase duration the interaction between temperature and pH was found to be insignificant ($p \leq 0.05$).

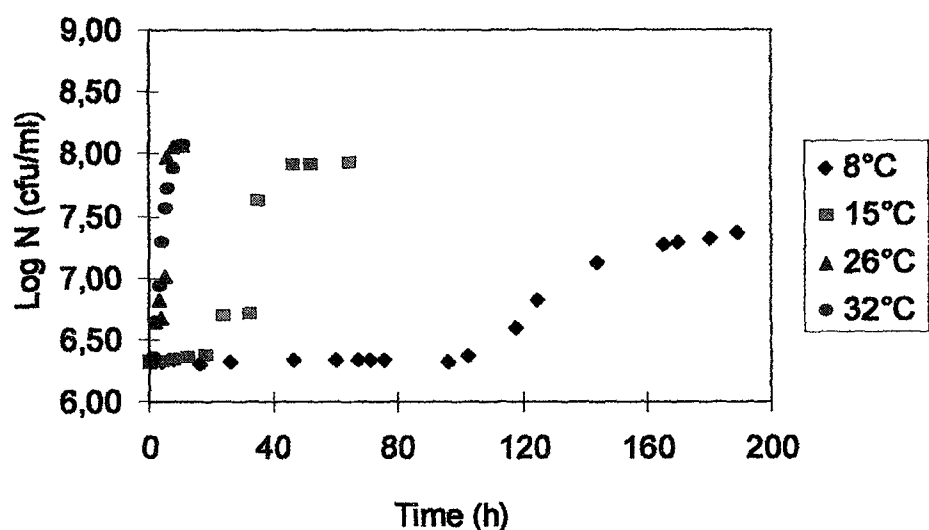


Figure 4. 10. Growth curves of *B. cereus* at different temperatures, at 7.3 pH, 200 mM sodium lactate and 85 mM sodium chloride.

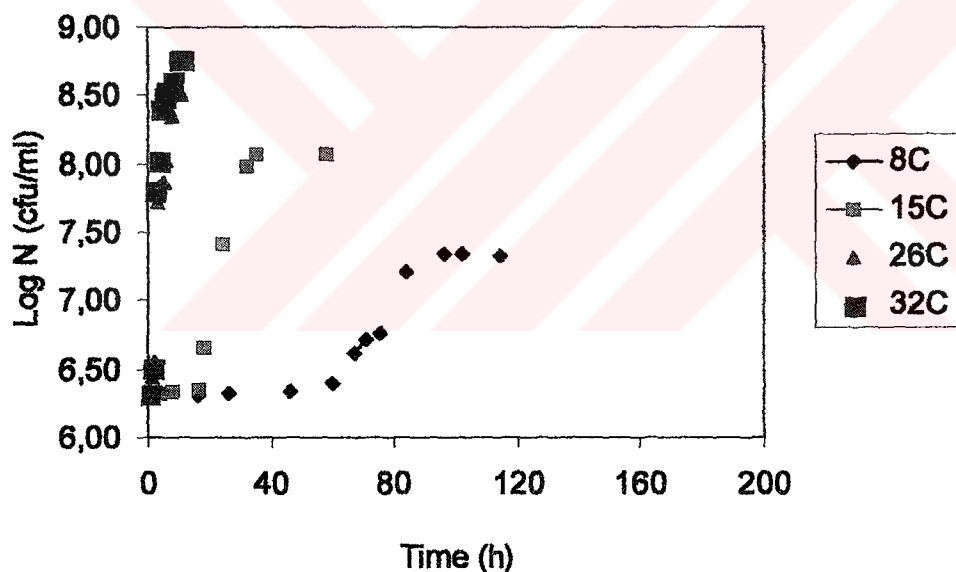


Figure 4. 11. Growth curves of *B. cereus* at different temperatures, at 6.8 pH, 0 mM sodium lactate and 85 mM sodium chloride.

Actually these types of factor interactions can be explained by the "hurdle effect". Adams and Moss (1995) explained that individual factors like temperature or pH, shown to be insufficient on their own to assure a significant decrease in growth rate or increase in lag phase duration, may cause significant changes in the growth kinetics of microorganisms when used in combination.

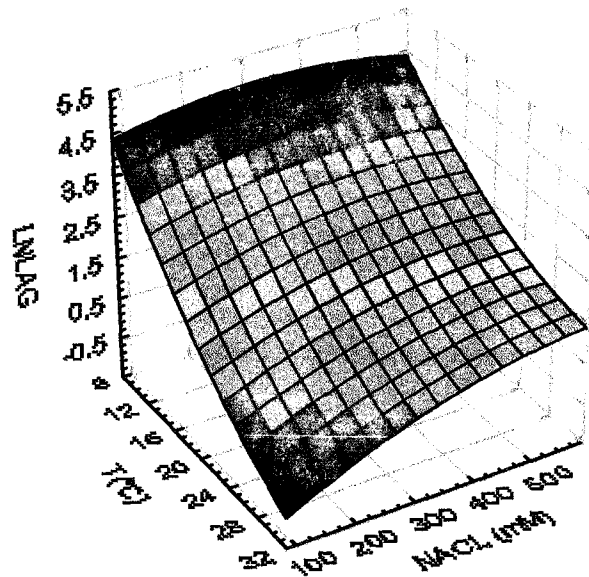


Figure 4. 12. Response surface graph showing the effect of temperature (T) and sodium chloride (NaCl) on the lag phase duration (LNLAG) of *B. cereus* at 6.3 pH, in the absence of sodium lactate.

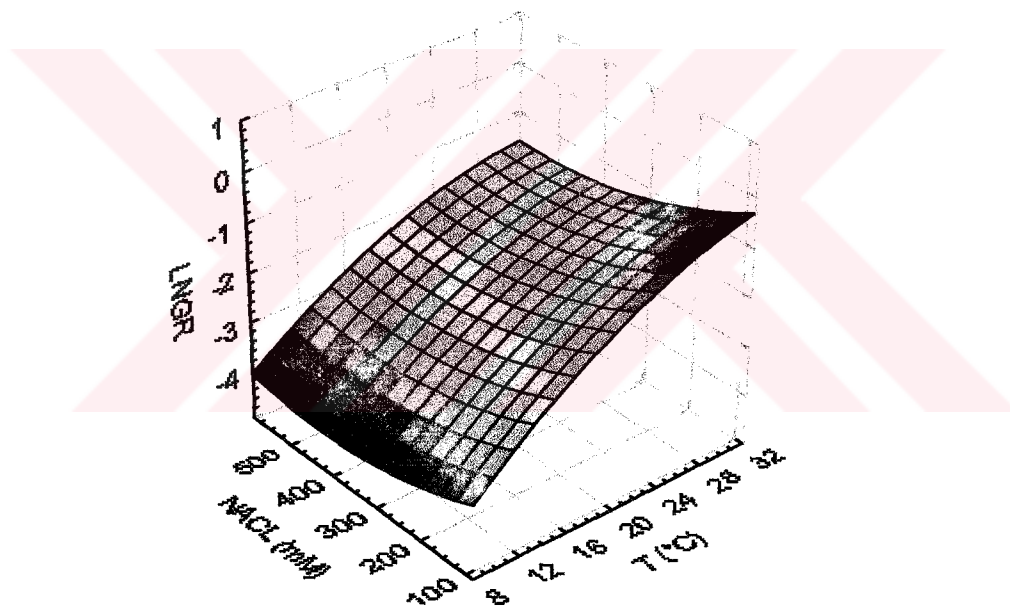


Figure 4. 13. Response surface graph showing the effect of temperature (T) and sodium chloride (NaCl) on the growth rate (LNGR) of *B. cereus* at 6.8 pH, in the absence of sodium lactate.

4. 6. Effect of pH on Lag Phase Duration (LAG) and Growth Rate (GR) of *B. cereus*

The results indicated that no growth was observed at a pH of 4.8 and growth occurred very slowly at the pH level 5.0 only at high temperatures (26-32°C) and in the presence of only 85 mM (% 0.5) NaCl. The minimum pH level for growth was determined to be between pH 4.8 to 5.0, which agrees well with the findings of

Roberts et al. (1996) for media acidulated with HCl. In Figure 4. 15, examples of growth curves at different pH levels are shown. It was observed that, although significant at a level of ($p \leq 0.05$), the effect of pH on the lag phase duration and growth rate of *B. cereus* was not as high as the effect of temperature, between the experimental range of the factors. The fitted polynomial model for $\ln GR$ and $\ln LAG$ (Table 4. 4) showed that the pH has a significant ($p \leq 0.05$) effect both on growth rate and lag phase duration in the studied experimental range. The growth was maximal at a pH of approximately 6.8, and minimal at a pH of 5.3.

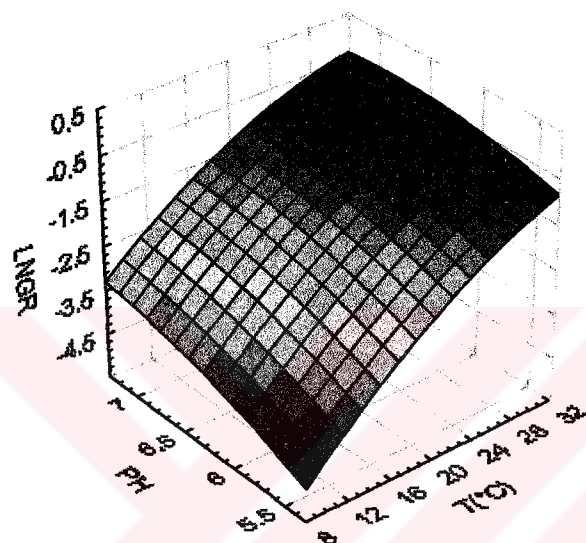


Figure 4. 14. Response surface graph showing the effect of temperature (T) and pH (PH) on the growth rate (LNGR) of *B. cereus* at 85 mM NaCl concentration, in the absence of sodium lactate.

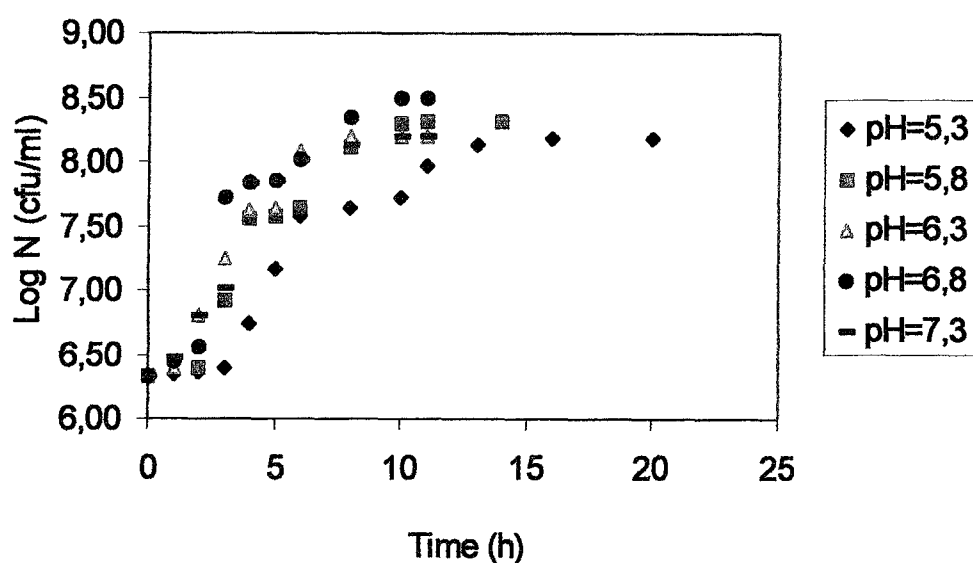


Figure 4. 15. Growth curves of *B. cereus* at different pH levels, at 26°C, 0 mM sodium lactate and 85 mM sodium chloride.

4. 7. Effect of Sodium Chloride on Lag Phase Duration (LAG) and Growth Rate (GR) of *B. cereus*

Sodium chloride concentrations between 85 mM (% 0.5) to 600 mM (% 3.5) were used in this study to be able to simulate real food systems. Examples of growth curves at different sodium chloride concentrations are shown in Figure 4. 16. In agreement with Benedict et al. (1993), it was found that sodium chloride has a significant ($p \leq 0.05$) effect both on growth rate and lag phase duration of *B. cereus* (Table 4. 4). The growth suppressing effect of sodium chloride increased with decreasing temperature (Figure 4. 13) and pH (Figure 4. 17). Moreover, the lag time prolonging effect of sodium chloride also increased with decreasing temperature (Figure 4. 12), whereas it was found to be unaffected from pH changes (Table 4. 4). Baker and Griffiths (1993) also stated that for the growth model of *B. cereus*, the interaction of temperature and water activity (adjusted by sodium chloride) was significant. The interaction between sodium chloride and other variables is an expected response, because the inhibitory effect of sodium chloride is attributed mainly to its water activity (a_w) lowering capacity and it is well known that the a_w requirements of microorganisms may be affected by temperature, pH, and the composition of the growth media (Frazier and Westhoff, 1988). Chorin et al. (1997) also observed that the growth rate of *B. cereus* increases to a greater extent with increasing pH at high a_w values than at lower a_w values. However, the effect of pH on lag phase duration of *B. cereus* was not affected from the changes in a_w .

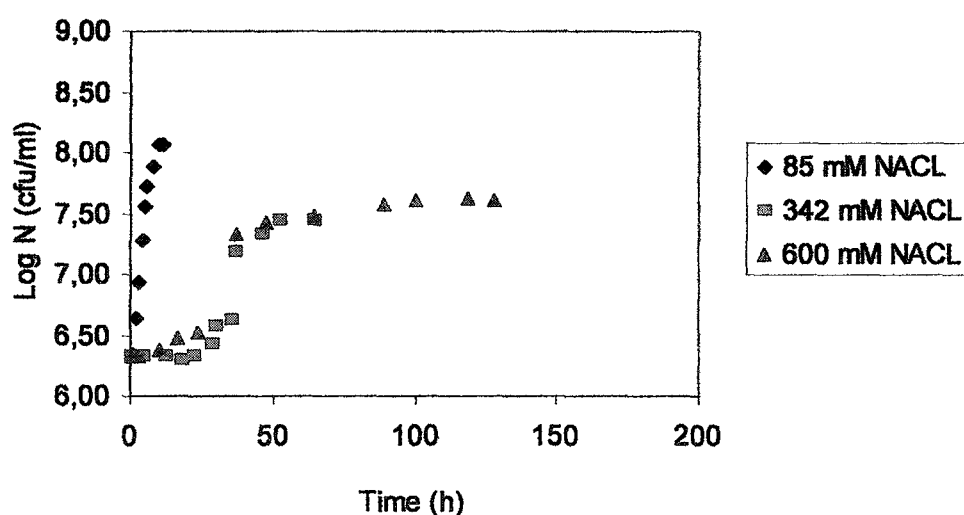


Figure 4. 16. Growth curves of *B. cereus* at different sodium chloride (NACL) levels, at 32°C, 200 mM sodium lactate and 7.3 pH.

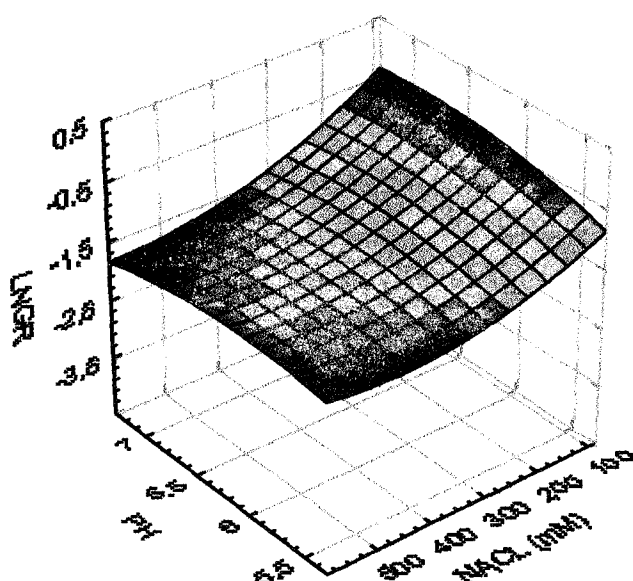


Figure 4. 17. Response surface graph showing the effect of sodium chloride concentration (NaCl) and pH (PH) on the growth rate (LNCR) of *B. cereus* at 32°C, in the absence of sodium lactate.

4. 8. Effect of Sodium Lactate on Lag Phase Duration (LAG) and Growth Rate (GR) of *B. cereus*

Examples of growth curves of *B. cereus* at different sodium lactate concentrations are given in Figure 4. 18 and 4. 19. At pH 5.3, no growth was observed even in the presence of only 200 mM sodium lactate. In addition, at 8°C, no growth was observed even at the lowest concentration of lactate, at the pH levels lower than 7.3. At a pH of 5.8, growth was not observed at lactate concentrations higher than 200 mM. Growth was inhibited at all temperatures, when 200 mM sodium lactate and 600 mM NaCl were used in combination. At 600 mM sodium lactate concentration, growth could only be observed when the pH was 7.3. Similarly, Houtsma et al. (1993) reported that the minimum inhibitory concentration (MIC) of sodium lactate for *B. cereus* is between 625-714 mM at pH 6.3 at 20°C. At 15°C and 26°C, growth was inhibited by 400 mM (% 3.5) sodium lactate at a pH of 5.8. In the presence of 342 mM (% 2) sodium chloride, 400 mM (% 3.5) sodium lactate was sufficient to inhibit growth at the pH level 6.3 at all temperature levels studied. Aran (2001) reported similar inhibitory levels for sodium lactate in beef goulash. At 20°C and 15°C, respectively % 3.0 and % 1.5 sodium lactate was found to inhibit growth of *B. cereus* in beef goulash containing % 1.6 sodium chloride at pH 6.0.

The linear effect of sodium lactate was found to be significant ($p \leq 0.05$) for In GR and In LAG models (Table 4. 4), whereas the second order terms were not. Moreover the interactions of sodium lactate with pH and temperature were also found to be significant for In LAG and In GR models, whereas no significant interaction was found between sodium lactate and sodium chloride neither for In LAG nor in GR models.

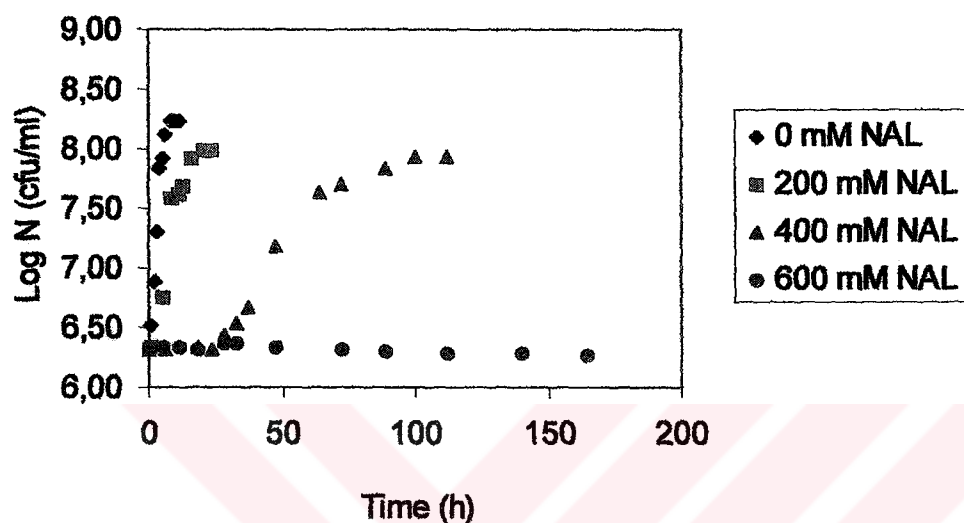


Figure 4. 18. Growth curves of *B. cereus* at different sodium lactate (NAL) levels, at 32°C, 85 mM sodium chloride and 6.3 pH.

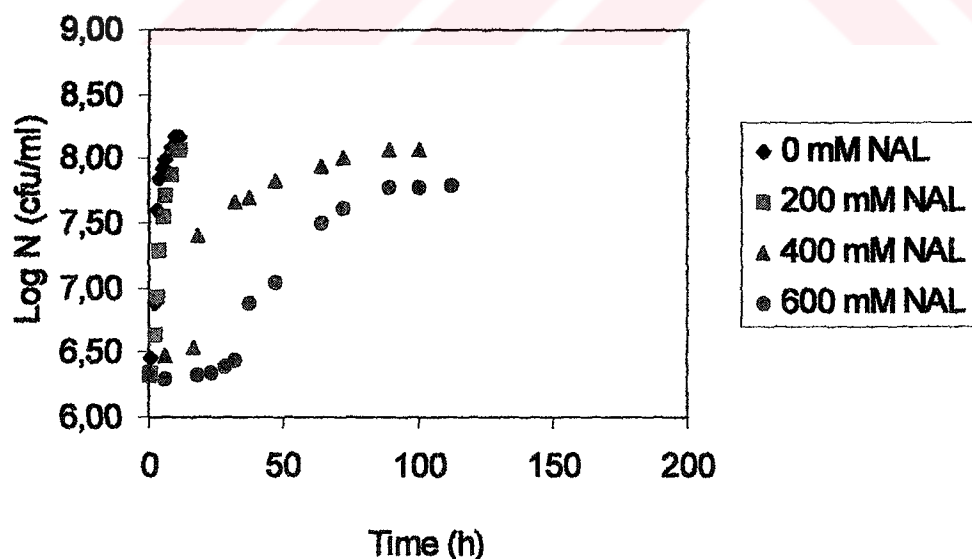


Figure 4. 19. Growth curves of *B. cereus* at different sodium lactate (NAL) levels, at 32°C, 85 mM sodium chloride and 7.3 pH.

As observed before by several researchers (Houtsma et al., 1996; Murphy et al., 1997; Blom et al., 1997), the bacteriostatic and bacteriocidal activities of sodium lactate for *B. cereus* were found to increase at low pH level. Less amount of sodium lactate was needed to inhibit the growth at acidic pH values. For example, 400 mM of sodium lactate was sufficient to inhibit the growth of *B. cereus* at pH 5.8, but 600 mM was needed at pH 6.3. In the same way, Houtsma et al. (1993) reported that, except for some lactic acid bacteria and *Salmonella*, the MIC values of sodium lactate for most of the bacteria studied decreased at low pH levels. Figure 4. 20 shows the effect of pH and sodium lactate on the lag phase duration of *B. cereus*. As shown in Figure 4. 20, at low pH values the increase in the lag phase duration of bacteria was higher than that of at higher pH levels. Moreover, in agreement with the observations of Grau (1980 and 1981) and Young et al. (1993), the decrease in growth rate was more pronounced at low pH levels than that of at high pH values (Figure 4. 21). The increase in the bacteriocidal activity of sodium lactate at low pH values may be the result of the decrease in intracellular pH of the bacterial cells (Cherrington et al., 1991; Salmond et al., 1984; Shelef, 1994). Because, Kaskhet (1987) stated that a decrease in intracellular pH results in a depression in cellular functions and below a certain level it may cause complete inhibition of cellular functions.

The interaction between temperature and sodium lactate was somewhat different from pH and should be treated carefully to avoid misunderstanding. It was seemed that, the antimicrobial activity of sodium lactate was higher at high temperatures, since the decrease in both the lag phase duration and growth rate was more evident at high temperatures with increasing sodium lactate concentrations (Figure 4. 22 and 4. 23). This observation contradicts with the observation of de Wit and Romboust (1990) who stated that suboptimum temperatures might increase the antimicrobial effect of sodium lactate. Moreover, Houtsma et al. (1996) reported that there is no specific effect of temperature on the activity of sodium lactate for most of the bacteria, except for *Debaryomyces hansenii*. This unexpected behavior might be a case specific to *B. cereus*, since it was reported that the specific effect of sodium lactate depends on how these microorganisms can cope with this compound by Houtsma et al. (1996) and de Wit and Rombouts (1990). It may be related with the changes in the cell membrane of this organism with changing temperature and how these changes in cell membrane affect the intake of substances like lactate into the cell. However, these discussions are beyond the scope of this study and they need to be investigated further by other studies.

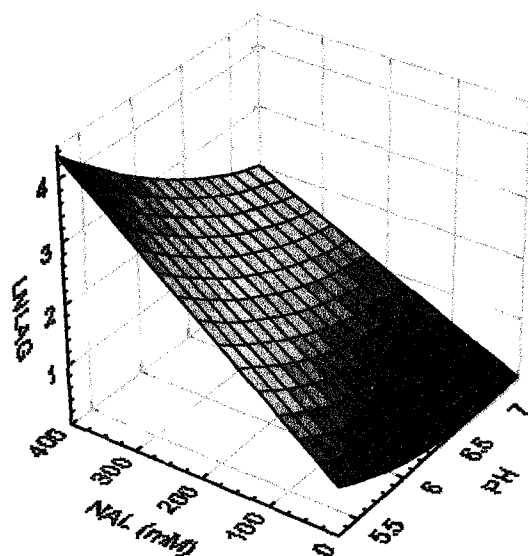


Figure 4. 20. Response surface graph showing the effect of sodium lactate concentration (NAL) and pH (PH) on the lag phase duration (LNLG) of *B. cereus* at 26°C, with 85 mM NaCl concentration.

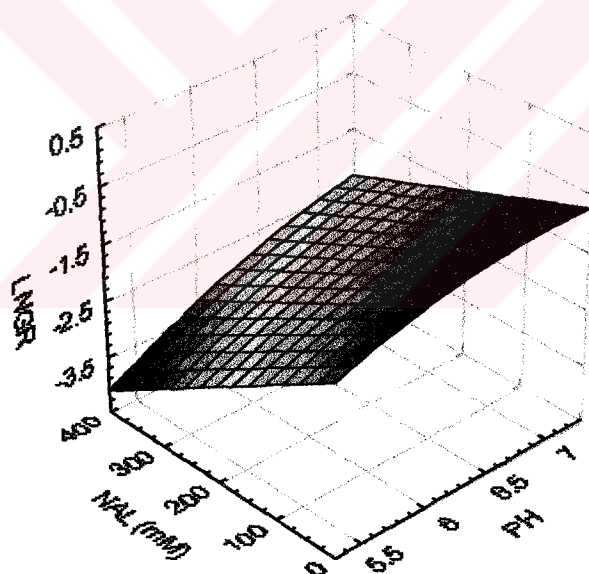


Figure 4. 21. Response surface graph showing the effect of sodium lactate concentration (NAL) and pH (PH) on the growth rate (LNGR) of *B. cereus* at 26°C, with 85 mM NaCl concentration.

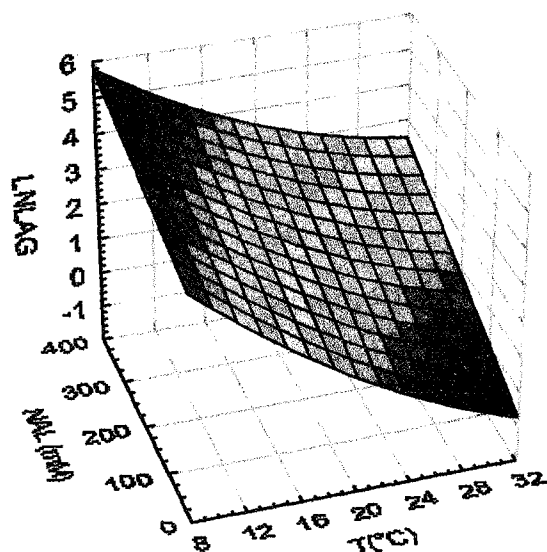


Figure 4. 22. Response surface graph showing the effect of sodium lactate concentration (NAL) and temperature (T) on the lag phase duration (LNLAG) of *B. cereus* at 6.3 pH, with 85 mM NaCl concentration.

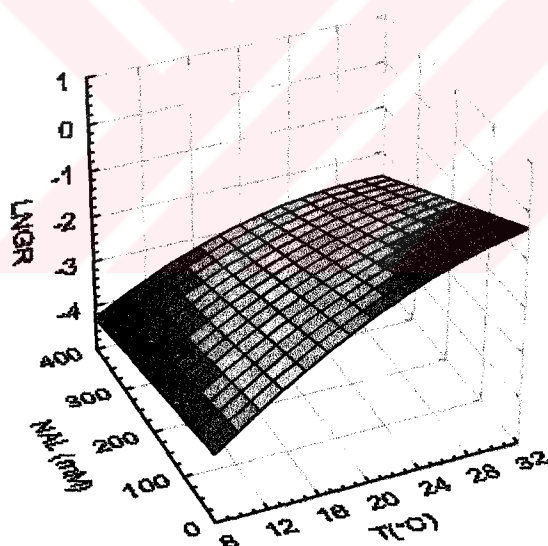


Figure 4. 23. Response surface graph showing the effect of sodium lactate concentration (NAL) and temperature (T) on the growth rate (LNCR) of *B. cereus* at 6.8 pH, with 85 mM NaCl concentration.

As shown in Table 4. 10, sodium lactate did not significantly reduce the water activity of the medium. Besides, at equal weight concentrations, sodium lactate was inferior in lowering the water activity of the medium compared to sodium chloride, as also reported by Chirife and Fontan (1980) and de Wit and Romboust (1990).

Therefore, it was concluded that, the inhibitory effect of sodium lactate could not be attributed to its water activity lowering capacity.

Table 4. 10. Water activity of the growth medium containing various concentrations of sodium chloride and sodium lactate at $21^{\circ}\text{C} \pm 0.34$.

NAL (%)	NaCl (%)	$a_{w\text{mean}} \pm \text{sd}$
0	0.5	0.997 ± 0.001
1.7	0.5	0.993 ± 0.001
3.5	0.5	0.990 ± 0.001
5.0	0.5	0.988 ± 0.001
0	3.5	0.981 ± 0.001
1.7	3.5	0.978 ± 0.001
5.0	3.5	0.976 ± 0.001

5. CONCLUSION AND FUTURE WORK

5. 1. Conclusion

Statistical analysis of the primary level and secondary level models showed that the Gompertz model was the best model describing the change in bacterial count of *B. cereus* with respect to time. The reduced In LAG and In GR models were best in describing the change in growth kinetic parameters of *B. cereus* with respect to temperature, pH, sodium chloride and sodium lactate concentrations.

Validation tests in milk and meat broth indicated that both the In LAG and In GR models provided safer growth estimates. In addition, validation of the In GR model against the literature data on *B. cereus* growth using generation times, showed that the predictions of the derived model were comparable with the predictions of PMP, and in some cases they were superior to PMP predictions. Almost in all cases, the In GR model provided safe estimates for generation time. Moreover, the MARE values of the derived model was found to be lower than most of the published models and PMP, indicating that it can be safely used for generation time (or growth rate) predictions.

The results revealed that sodium lactate displayed both bacteriostatic and bactericidal activity against *B. cereus*. Bacteriostasis typically involved both decreased EGR and extended LPD. Bactericidal activity was limited largely to a slow die-off of non-growing cultures in association with higher sodium lactate levels. The antimicrobial activity of sodium lactate was highly dependent on the initial pH of the medium, and to a smaller extent on the temperature. However, no significant interaction was found between the antimicrobial activity of sodium lactate and sodium chloride. It was shown that, at the pH levels lower than 7.3, 600 mM sodium lactate was sufficient to inhibit the growth of *B. cereus* irrespective of the temperature.

5. 2. Future Work

The mechanism of the specific antimicrobial activity of sodium lactate on *B. cereus* need to be studied in detail. Moreover, the effect of pH and temperature on the antimicrobial activity of sodium lactate should also be investigated further.

Since there were not enough data about the growth kinetics of *B. cereus* in the presence of sodium lactate in the literature, the validation of the models was limited to the cases in which sodium lactate was not present. In addition, the validation tests in meat broth supported only very limited knowledge about the efficiency of the derived models in estimating the effect of sodium lactate on the growth kinetic parameters of *B. cereus*. Therefore, in relation to the published results, the real performance of the models in predicting the growth behavior of *B. cereus* depending on the sodium lactate concentration could not be evaluated in this study. This should be the subject of a future study in which the effect of various levels of sodium lactate on the growth kinetics of *B. cereus* can be investigated in various foods with different pH levels and compositions that are stored at different temperatures. Moreover, the interaction between sodium lactate and other food preservatives like nisin, potassium sorbate or sodium benzoate, also worth studying. Because, the results of such a study may provide information on the efficacy of sodium lactate against *B. cereus* in combination systems, and may enable the use of more effective and safer preservative combinations and thereby formulation of safer food products.

REFERENCES

- Adams, M.R. and Moss, M.O., 1995. Food Microbiology, The Royal Society of Chemistry, Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK.
- Adams, M.R., Little, C.L. and Easter, M.C., 1991. Modelling the effect of pH, acidulant and temperature in the growth rate of *Yersinia enterocolitica*, *J. Appl. Bacteriol.*, **71**, 65-71.
- Aran, N., 2001. The effect of calcium and sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a 'sous vide' beef goulash under temperature abuse, *Int. J. Food Microbiol.*, **63**, 117-123.
- Asplund, K., Nurmi, E., Hill, P. and Hirn, J., 1988. The inhibition of the growth of *Bacillus cereus* in liver sausage, *Int. J. Food Microbiol.*, **7**, 349-352.
- Baird-Parker, A.C. and Kilsby, D.C., 1987. Principles of predictive food microbiology, *J. Appl. Bacteriol. Symposium Supplement* 43S-49S.
- Baker, J. M. and Griffiths, M. W., 1993. Predictive modeling of psychrotrophic *B. cereus*, *J. Food Protection*, **56** (8), 684-688.
- Baranyi, J and Roberts, T.A., 1995. Mathematics of predictive food microbiology, *Int. J. Food Microbiol.*, **26**, 199-218.
- Baranyi, J., McClure, P.J., Sutherland, J.P. ve Roberts, T.A., 1993. Modeling bacterial growth responses, *J. Ind. Microbiol.*, **12**, 190-194.
- Baranyi, J., Ross, T., McMeekin, T.A. and Roberts, T.A., 1996. Effects of parametrisation on the performance of empirical models used in predictive microbiology, *Food Microbiol.*, **13**, 83-91.
- Bělehrádek, J., 1930. Temperature coefficients in biology, *Biol. Rev. Biol. Proc. Cambridge Philos. Soc.*, **5**, 30-58.
- Benedict, R. C., Partridge, T., Wells, D. and Buchanan, R. L., 1993. *Bacillus cereus*: Aerobic growth kinetics, *J. Food Protection*, **56** (3), 211-214.
- Bhaduri, S., Turner-Jones, C.O., Buchanan, R.L. and Phillips, J.G., 1994. Response surface model of the effect of pH, sodium chloride and sodium nitrite on growth of *Yersinia enterocolitica* at low temperatures, *Int. J. Food Microbiol.*, **23**, 333-343.
- Blakey, J. J. and Priest, F. G., 1980. The occurrence of *Bacillus cereus* in some dried foods including pulses and cereals, *J. Appl. Bacteriol.*, **48**, 297-302.

- Blom, H., Nerbrink, E., Dainty, R., Hagtvedt, T., Borch, E., Nissen, H., and Nesbakken, T., 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory acceptable served sausage and cooked ham stored at 4°C, *Int. J. Food Microbiol.*, **38**, 71-76.
- Borge, G. I. A., Skeie, M., Sørhaug, T., Langsrud, T., and Granum, P. E., 2001. Growth and toxin profiles of *Bacillus cereus* isolated from different food sources, *Int. J. Food Microbiol.*, **69**, 237-246.
- Box, G.E.P. and Draper, N.R., 1987. Empirical Model-Building and Response Surfaces, John Wiley and Sons, New York.
- Buchanan, R.L., 1993. Developing and distributing user-friendly application software, *J. Ind. Microbiol.*, **12**, 251-255.
- Buchanan, R.L. and Klawitter, L.A., 1991. Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperatures, *Int. J. Food Microbiol.*, **12**, 235-246.
- Buchanan, R.L. and Phillips, J.G., 1990. Response surface model for predicting the effect of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*, *J. Food Protection*, **53**, 370-376.
- Buchanan, R.L., Bagi, L.K., Goins, R.V. and Phillips, J.G., 1993. Response surface models for the growth kinetics of *Escherichia coli* O157:H7, *Food Microbiol.*, **10**, 303-315.
- Buchanan, R.L., Smith, J.L., McColgan, C., Marmer, B.S., Golden, M. and Dell, B., 1993. Response surface models for the effects of temperature, pH, sodium chloride, and sodium nitrite on the aerobic and anaerobic growth of *Staphylococcus aureus* 196E, *J. Food Safety*, **13**, 159-175.
- Cherrington, C.A., Hinton, M., Mead, G.C. and Chopra, I., 1991. Organic acids: chemistry, antibacterial activity and practical applications, in *Advances in Microbial Physiology*, pp. 87-108, Eds. Rose, A.H. and Tempest, D.W., Academic Press, London.
- Chirife, J. and Fontan, C. F., 1980. Prediction of water activity of aqueous solutions in connection with intermediate moisture foods: experimental investigation of the a_w lowering behaviour of sodium lactate and some related compounds, *J. Food Sci.*, **45**, 802-804.
- Christianson, A., Naidu, A.S., Nilsson, I., Wadstrom, T. and Pettersson, H., 1989. Toxin production by *Bacillus cereus* dairy isolates in milk at low temperatures, *App. and Environ. Microbiol.*, **55**(10), 2595-2600.
- Choma, C., Clavel, T., Dominguez, H., Razandramboa, N., Soumille, H., Nguyenthe, C., and Schmitt, P., 2000. Effect of temperature on growth characteristics of *Bacillus cereus* TZ415, *Int. J. Food Microbiol.* **55**, 73-77.
- Chorin, E., Thuault, D., Cleret, J. J. and Bourgeois, C. M., 1997. Modelling *B. cereus* growth, *Int. J. Food Microbiol.*, **38**, 229-234.

- Davey, K.R., 1989. A predictive model for combined temperature and water activity on microbial growth during the growth phase, *J. Appl. Bacteriol.*, **67**, 483-488.
- Davey, K.R., 1991. Applicability of the Davey (linear Arrhenius) predictive model to the lag phase of microbial growth, *J. Appl. Bacteriol.*, **70**, 253-257.
- Davies, K.W., 1993. Design of experiments for predictive microbial modelling, *J. Ind. Microbiol.*, **12**, 295-300.
- Delignette-Muller, M.L., Rosso, L. and Flandrois, J.P., 1995. Accuracy of microbial growth predictions with square root and polynomial models, *Int. J. Food Microbiol.*, **27**, 139-146.
- de Raat, H. and van Burik, A., 1990. Laktaten van natuurlijk L(+)-melkzuur toegepast om bederf van levensmiddelen tegen te gaan, *Voedingsmiddelentechnologie*, **23**, 28-30.
- de Wit, J.C. and Romboust, F.M., 1990. Antimicrobial activity of sodium lactate, *Food Microbiol.*, **7**, 113-120.
- Doyle, M.P., 1988. Effect of environmental and processing conditions on *Listeria monocytogenes*. *Food Technol.*, **42**, 169-171.
- Drobroniewski, F.A., 1993. *Bacillus cereus* and related species, *Clinical Microbiol. Rev.*, **6**(4), 324-338.
- Dufrenne, J., Soentoro, P., Tatini, S., Day, T. and Notermans, S., 1994. Characteristics of *Bacillus cereus* related to safe food production, *Int. J. Food Microbiol.*, **23**, 99-109.
- Dufrenne, J., Bijwaard, M., te Giffel, M., Beumer, and R., Notermans, S., 1995. Characteristics of some psychrotrophic *Bacillus cereus* isolates, *Int. J. Food Microbiol.*, **27**, 175-183.
- Duxbury, D.D., 1988. Natural sodium lactate extends shelf-life of whole and ground meats, *Food Process.*, Jan., 91.
- Eifert, J.D., Gennings, C., Carter, W.H., Duncan, S.E. and Hackney, C.R., 1996. Predictive model with improved statistical analysis of interactive factors affecting growth of *Staphylococcus aureus* 196E, *J. Food Protection*, **59** (6), 608-614.
- Eklund, T., 1989. Organic acids and esters, in *Mechanism of Action of Food Preservation Procedures*, pp. 161-200, Eds. G.W. Gould, Elsevier Applied Science, London.
- Fernandez, P.S., George, S.M., Sills, C.C. and Peck, M.W., 1997. Predictive model of the effect of CO₂, pH, temperature and NaCl on the growth of *Listeria monocytogenes*, *Int. J. Food Microbiol.*, **37**, 37-45.
- Frazier, W. C. and Westhoff, D. C., 1988. *Food Microbiology*, International Edition, McGraw Hill Book Co., Singapore.

- Garcia-Arribas, M.L. and Kramer, J.M., 1990.** The effect of glucose, starch, and pH on growth, enterotoxin and haemolysin production by strains of *Bacillus cereus* associated with food poisoning and non-gastrointestinal infection. *Int. J. Food Microbiol.*, **11**, 21-34.
- Garcia-Arribas, M.L., Plaza, C.L., De La Rosa, M.C. and Mosso, M.A., 1988.** Characterisation of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins, *J. Appl. Bacteriol.*, **64**, 257-264.
- Gartricht, W.E., 1991.** Refinements in the prediction of microbial growth curves. *Food Microbiol.*, **8**, 239-248.
- Gibson, A.M., Bratchell, N. and Robets, 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.
- Gibson, A.M., Bratchell, N. and Roberts, T.A., 1988.** Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature, *Int. J. Food Microbiol.*, **6**, 155-178.
- Gilbert, R. J., Stringer, M. F. and Peace, T. C., 1974.** The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning, *J. Hyg.*, **74**, 433-444.
- Gompertz, B., 1825.** On the nature of the function of expressive of the law of human mortality, and on a new mode of determining the value of life contingencies, *Philos. Trans. R. Soc. London*, **115**, 513-585.
- Granum, P.E., Brynestad, S. and Kramer, J., 1993.** Analysis of enterotoxin production by *Bacillus cereus* from dairy products, food poisoning incidents and non-gastrointestinal infections, *Int. J. Food Microbiol.*, **17**, 262-279.
- Granum, P. E., 1994.** *Bacillus cereus* and its toxins, *J. Appl. Bacteriol.*, **76** (Symposium Supplement), 61S-66S.
- Grau, F.H., 1980.** Inhibition of the anaerobic growth of *Brochotrix thermosphacta* by lactic acid, *Appl. Environ. Microbiol.*, **40**, 433-436.
- Grau, F.H., 1981.** Role of pH, lactate, and anaerobiosis in controlling the growth of some fermentative gram-negative bacteria on beef, *Appl. Environ. Microbiol.*, **42**, 1043-1050.
- Greer, G.C. and Dilts, B.D., 1992.** Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids, *Food Res. Int.*, **25**, 355-364.
- Harmon, S.M. and Kautter, D. A., 1991.** Incidence and growth potential of *Bacillus cereus* in ready-to-serve foods, *J. Food Protection*, **54**(5), 372-374.
- Hauschild, A.H.W., 1982.** Assessment of botulism hazards from cured meat products, *Food Technol.*, **36**, 95-104.

- Holbrook, R. and Anderson, J. M., 1980. An improved selective and diagnostic medium for the isolation and enumeration of *Bacillus cereus* in foods, *Can. J. Microbiol.*, **26**, 753-759.
- Houtsma, P.C., Kant-Muermans, M.L., Romboust, F.M. and Zwietering, M.H., 1996. Model for the combined effects of temperature, pH, and sodium lactate on growth rates of *Listeria innocua* in broth and bologna-type sausages, *Appl. Environ. Microbiol.*, **62**, 1616-1622.
- Houtsma, P. C., Wit, J. C. and Rombouts, F. M., 1993. Minimum inhibitory concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring in meat products, *Int. J. Food Microbiol.*, **20** (4), 247-257.
- Hudson, J.A., 1994. Comparison of response surface models for *Listeria monocytogenes* strains under aerobic conditions, *Food Res. Int.*, **27**, 53-59.
- ICMSF, 1996. *Bacillus cereus*, in *Microorganisms in Foods*, pp. 20-35. London: Blackie Academic & Professional.
- Jakobsen, M., Filtenborg, O. and Bramsnaes, F., 1972. Germination and outgrowth of the bacterial spore in the presence of different solutes, *Lebensm. Wiss. Technol.*, **5**, 159-162.
- Jaquette, C.B. and Beuchat, L.B., 1998. Survival and growth of psychrotrophic *Bacillus cereus* in dry and reconstituted infant rice cereal, *Journal of Food Protection*, **61**(12), 1629-1635.
- Johnson, K.M., Nelson, C.L. and Busta, F.F., 1983. Influence of temperature on germination and growth of spores of emetic and diarrheal strains of *Bacillus cereus* in a broth medium and in rice, *J. Food Sci.*, **48**, 286-287.
- Kashket, E.R., 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance, *FEMS Microbiol. Rev.*, **46**, 233-244.
- Keay, L. and Wildi, B.S., 1970. Proteases of the genus *Bacillus*. I. Neutral proteases, *Biotechnol. Bioeng.*, **12**, 179-212.
- Kramer, J. M. and Gilbert, R. J., 1989. *Bacillus cereus* and other *Bacillus* species, in *Foodborne Bacterial Pathogens*, pp. 21-70, Eds. Doyle, M. P., New York: Marcel Dekker.
- Lancette, G.A. and Harmon, S.M., 1980. Enumeration and confirmation of *Bacillus cereus* in foods: collaborative study, *J. AOAC Int.*, **63**, 581-586.
- Maas, M.R., Glass, K.A. and Doyle, M.P., 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products, *Appl. Environ. Microbiol.*, **55**, 2226-2229.
- Marshall, B.J., Ohye, D.F. and Christian, J.H.B., 1971. Tolerance of bacteria to high concentrations of NaCl and glycerol in the growth medium, *Appl. Microbiol.*, **21**, 363-364.

- McClure, P.J., Baranyi, J., Boogard, E., Kelly, T.M. and Roberts, T.A., 1993.** A predictive model for the combined effect of pH, sodium chloride and storage temperature on the growth of *Brochothrix thermosphacta*, *Int. J. Food Microbiol.*, **19**, 161-178.
- McClure, P.J., Blackburn, C.W., Cole, M.B., Curtis, P.S., Jones, J.E., Legan, J.D., Ogden, I.D., Peck, M.W., Roberts, T.A., Sutherland, J.P. and Walker, S.J., 1994.** Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach, *Int. J. Food Microbiol.*, **23**, 265-275.
- McMeekin, T.A., Brown, J., Krist, K., Miles, D., Neumeyer, K., Nichols, D.S., Olley, J., Presser, K., Ratkowsky, D.A., Ross, T., Salter, M., and Soontranon, S., 1997.** Quantitative microbiology: a basis for food safety, *Emerging Infect. Dis.*, **3**, 541-549.
- McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putros, S. and Ratkowsky, D.A., 1987.** Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus*, *J. Appl. Bacteriol.*, **62**, 543-550.
- McMeekin, T. A., Olley, J.N., Ross, T. and Ratkowsky, D. A., 1993.** Introduction. In: *Predictive Microbiology: Theory and Application*, pp.1-9. Research Studies Press Ltd./John Wiley and Sons Inc. New York.
- McMeekin, T. A., Olley, J.N., Ratkowsky, D. A. and Ross, T., 2002.** Predictive microbiology: towards the interface and beyond, *Int. J. Food Microbiol.*, **73**, 395-407.
- McMeekin, T. A., Presser, K., Ratkowsky, D. A., Ross, T., Salter, M. and Tienungoon, S., 2000.** Quantifying the hurdle concept by modelling the bacterial growth/no growth interface, *Int. J. Food Microbiol.*, **55**, 93-98.
- McMeekin, T.A., Ross, T. and Olley, J., 1992.** Application of predictive microbiology to assure the quality and safety of fish and fish products. *Int. J. Food Microbiol.*, **15**, 13-32.
- McMeekin, T.A. and Ross, T., 2002.** Predictive microbiology: providing a knowledge-based framework for change management, *Int. J. Food Microbiol.*, **78**, 133-153.
- McNab, W.B., 1997.** A literature review linking microbial risk assessment, predictive microbiology, and dose-response modeling, *Dairy, Food and Environ. Sanit.*, **17** (7), 405-416.
- Measures, J., 1975.** Role of amino acids in osmo regulation of nonhalophilic bacteria, *Nature* (London), **257** (5525), 398-399.
- Meer, R.R., Baker, J., Bodyfelt, F.W. and Griffiths, M.W., 1991.** Psychrotrophic *Bacillus* spp. in Fluid Milk Products: A Review, *J. Food Protection*, **54**(12), 969-979.
- Mossel, D.A.A., Koopman, M.J. and Jongerius, E., 1967.** Enumeration of *Bacillus cereus* in foods, *Appl. Microbiol.*, **15**, 650-653.

- Murphy, P.M., Rea, M.C. and Marchand, E., 1997.** Predicting bacterial growth in processed milk and dairy products, *IIF-IIR-Commission C2*, Quimper, France, 1997/2, 213-223.
- Neidhardt, F. C., Ingraham, J. L. and Schaechter, M. 1990.** Physiology of Bacterial Cell, Sinauer Associates, Inc. Massachusetts, USA.
- Nester, S. and Woodburn, M., 1982.** Contamination and growth of *Bacillus cereus* and *Clostridium perfringens* in Mexican-style beans, *J. Food Protection*, **45**, 638-642.
- Notermans, S. and Batt, C.A., 1998.** A risk assessment approach for food-borne *Bacillus cereus* and its toxins, *J. Appl. Bacteriol. Symposium Supplement*, **84**, 51S-61S.
- Ohta, F. and Hirahara, T., 1977.** Rate of degradation in cool-stored carp muscle, *Mem. Fac. Fish. Kagoshima Univ.*, **26**, 97-102.
- Olley, J., 1986.** In defence of library budgets: a tribute to two librarians, *Food Technol. Aust.*, **38**, 276-280.
- Oscar, T.P., 1999.** Response surface models for the effects of temperature, pH and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth, *J. Food Protection*, **62** (2), 106-111.
- Palumbo, S.A., Williams, A.C., Buchanan, R.L. and Phillips, J.G., 1991.** Model for the aerobic growth of *Aeromonas hydrophila* K144, *J. Food Protection.*, **5**, 429-435.
- Palumbo, S.A., Williams, A.C., Buchanan, R.L. and Phillips, J.G., 1992.** Model for the anaerobic growth of *Aeromonas hydrophila* K144, *J. Food Protection.*, **4**, 260-265.
- Papadopoulos, L.S., Miller, R.K., Acuff, G.R., Vanderzant, C. and Cross, H.R., 1991.** Effect of sodium lactate on microbial and chemical composition of cooked beef during storage, *J. Food Sci.*, **56**, 341-347.
- Presser, K. A., Ratkowsky, D. A., and Ross, T., 1997.** Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration, *Appl. Environ. Microbiol.*, **63** (6), 2355-2360.
- Quintavalla, S. and Parolari, G., 1993.** Effects of temperature, water activity and pH on the growth of *Bacillus* cells and spores: a response surface methodology study, *Int. J. Food Microbiol.*, **19**, 207-216.
- Qvist, S., Sehested, K. and Zeuhten, P., 1994.** Growth suppression of *Listeria monocytogenes* in a meat product, *Int. J. Food Microbiol.*, **24**, 283-293.
- Rajkowski, K.T. and Mikolajcik, E.M., 1987.** Characteristics of selected strains of *Bacillus cereus*, *J. Food Protection*, **50** (3), 199-205.
- Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stroke, A.N. and Chandler, R.E., 1983.** Model for bacterial growth rate throughout the entire biokinetic temperature range, *J. of Bacteriol.*, **154**, 1222-1226.

- Ratkowsky, D.A., Ross, T., McMeekin, T.A. and Olley, J., 1991.** Comparison of Arrhenius-type and Bělehrádek-type models for prediction of bacterial growth in foods, *J. Appl. Bacteriol.*, **71**, 452-459.
- Richards, F.J., 1959.** A flexible growth function for empirical use, *J. Exp. Bot.*, **10**, 290-300.
- Roberts, T.A., 1989.** Combinations of antimicrobials and processing methods. *Food Technol.*, **43**, 156-163.
- Rodriguez, M. and Barret, E., 1986.** Changes in microbial population and growth of *Bacillus cereus* during storage of reconstituted dry milk, *J. Food Prot.*, **49**, 680-686.
- Ross, T., 1987.** Bělehrádek temperature functions and growth of organisms, *CSIRO-DSIR Joint Workshop on Seafood Processing*, Nelson, New Zealand, April 1986. Occasional Paper 18, CSIRO Tasmanian Regional Laboratory, Hobart, Australia, 16 pp.
- Ross, T. and McMeekin, T.A. 1991.** Predictive microbiology-Applications of a square root model. *Food Australia* **43** 202-207.
- Ross, T. and McMeekin, T.A., 1994.** Predictive microbiology, *Int. J. Food Microbiol.*, **23**, 241-264.
- Russel, J.B., 1992.** A review: Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling, *J. Appl. Bacteriol.*, **73**, 363-370.
- Salmond, C.V., Kroll, R.G. and Booth, I.R., 1984.** The effects of food preservatives on pH homeostatis in *Escherichia coli*, *J. Gen. Microbiol.*, **130**, 2845-2850.
- Schnute, J., 1981.** A versatile growth model with statistically stable parameters, *Can. J. Fish. Aquat. Sci.*, **38**, 1128-1140.
- Shelef, L. A., 1994.** Antimicrobial effects of lactates: A review, *J. Food Protection*, **57** (5), 445-450.
- Skinner, G.E, Larkin, J.W. and Rhodehamel, E.J., 1994.** Mathematical modeling of microbial growth: a review, *J. Food Safety*, **14**, 175-217.
- Skinner, G.E and Larkin, J.W., 1994.** Mathematical modeling of microbial growth: a review, *J. Food Safety*, **14**, 175-217.
- Soboleva, T.K., Pleasants, A.B. and le Roux, G., 2000.** Predictive microbiology and food safety, *Int. J. Food Microbiol.*, **57**, 183-192.
- Sooltan, J.R.A., Mead, C.G. and Norris, A.P., 1987.** Incidence and growth potential of *Bacillus cereus* in poultry meat products. *Food Microbiol.*, **4**, 347-351.
- Stannard, C.J., Williams, A.P. and Gibbs, P.A., 1985.** Temperature/growth relationship for psychrotrophic food-spoilage bacteria. *Food Microbiol.*, **2**, 115-122.

- Sutherland, J.P., Bayliss, A.J. and Roberts, T.A., 1994.** Predictive modelling of growth of *Staphylococcus aureus*: the effects of temperature, pH, and sodium chloride, *Int. J. Food Microbiol.*, **21**, 217-236.
- Sutherland, J. P., Aherne, A. and Beaumont, A. L., 1996.** Preparation and validation of a growth model for *B. cereus*: the effects of temperature, pH, sodium chloride and carbon dioxide, *Int. J. Food Microbiol.*, **30**, 359-372.
- Tan, W. and Shelef, L.A., 2002.** Effects of sodium chloride and lactates on chemical and microbiological changes in refrigerated and frozen fresh ground pork, *Meat Sci.*, **62**, 27-32.
- Ten Brink, B., Otto, R., Hansen, U.P. and Konings, W.N., 1985.** Energy recycling by lactate efflux in growing and nongrowing cells of *Streptococcus cremoris*, *J. Bacteriol.*, **162**, 383-390.
- Tinoco, I., Sauer, K., and Wang, J. C., 1978.** Physical Chemistry. Prentice-Hall, Inc., Engelwood Clifs, N.J., USA.
- Unda, J.R., Molins, R.A. and Walker, H.W., 1991.** *Clostridium sporogenes* and *Listeria monocytogenes*: survival and inhibition in microwave-ready beef roasts containing selected antimicrobials, *J. Food Sci.*, **56**, 198-205.
- USDA/ARS 1997.** Pathogen Modeling Program, version 5.0, Microbial Food Safety Research Unit, USDA/ARS Eastern Regional Research Center, Wyndmoor, Pa.
- Van Netten, P., van de Moosdijk, A., van Hoensel, D., Mossel, D.A.A. and Perales, I., 1990.** Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. *J. Appl. Bacteriol.*, **69**, 73-79.
- Walker, S.J., Archer, P. and Banks, J.G., 1990.** Growth of *Listeria monocytogenes* at refrigeration temperatures, *J. Appl. Bacteriol.*, **68**, 157-162.
- Walker, S.J. and Jones, J.E., 1993.** Protocols for data generation for predictive modeling, *J. Ind. Microbiol.*, **12**, 273-276.
- Weaver, R.A. and Shelef, L.A., 1993.** Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage, *J. Food Safety*, **13**, 133-1461.
- Whiting, R.C. and Buchanan, R.L., 1994.** Microbial Modeling, *Food Technol.*, **48** (6), 113-120.
- Wijtzes, T., McClure, P.J., Zwietering, M.H. and Roberts, T.A., 1993.** Modelling bacterial growth of *Listeria monocytogenes* as a function of acidity and temperature, *Appl. Environ. Microbiol.*, **61**, 2533-2539.
- Wong, H.C. and Chen, Y.L., 1988.** Effects of lactic acid bacteria and organic acids on growth and germination of *Bacillus cereus*, *Appl. Environ. Microbiol.*, **54**, 2179-2184.

- Wong, H.C., Chang, M.A. and Fan, J.Y., 1988.** Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products, *Appl. Environ. Microbiol.*, **54**, 699-702.
- Young, K.M. and Foegeding, P.M., 1993.** Acetic, lactic and citric acids and pH inhibition of *Listeria monocytogenes* Scott A and the effect on intracellular pH, *J. Appl. Bacteriol.*, **74**, 515-520.
- Zaika, L.L., Phillips, J.G. and Buchanan, R.L., 1992.** Model for aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride and sodium nitrite concentrations, *J. Food Prot.*, **55**, 509-513.
- Zwietering, M. H., Jongenburger, I., Rombouts, F. M. and Van't Riet, K., 1990.** Modeling of the bacterial growth curve, *Appl. Environ. Microbiol.*, **56**, 1875-1881.



APPENDICES



APPENDIX A. EXPERIMENTAL DESIGN

Table A. 1. Fractional factorial design.

No	T (°C)	pH	NAL (mM)	NaCl (mM)
1	32	6.3	0	85
2	32	5.3	0	85
3	32	6.3	0	600
4	32	5.3	0	600
5	32	6.3	400	85
6	32	5.3	400	85
7	32	6.3	400	600
8	32	5.3	400	600
9	8	6.3	0	85
10	8	5.3	0	85
11	8	6.3	0	600
12	8	5.3	0	600
13	8	6.3	400	85
14	8	5.3	400	85
15	8	6.3	400	600
16	8	5.3	400	600

Table A. 2. Full factorial design.

No	T (°C)	pH	NaL (mM)	NaCl (mM)
1	32	5,3	0	85
2	32	5,3	200	85
3	32	5,3	400	85
4	32	5,3	600	85
5	32	5,3	0	600
6	32	5,3	200	600
7	32	5,3	400	600
8	32	5,3	600	600
9	32	6,3	0	85
10	32	6,3	200	85
11	32	6,3	400	85
12	32	6,3	600	85
13	32	6,3	0	600
14	32	6,3	200	600
15	32	6,3	400	600
16	32	6,3	600	600
17	32	7,3	0	85
18	32	7,3	200	85
19	32	7,3	400	85
20	32	7,3	600	85
21	32	7,3	0	600
22	32	7,3	200	600
23	32	7,3	400	600
24	32	7,3	600	600
25	26	5,3	0	85
26	26	5,3	200	85
27	26	5,3	400	85
28	26	5,3	600	85
29	26	5,3	0	600
30	26	5,3	200	600
31	26	5,3	400	600
32	26	5,3	600	600
33	26	6,3	0	85
34	26	6,3	200	85
35	26	6,3	400	85
36	26	6,3	600	85
37	26	6,3	0	600
38	26	6,3	200	600
39	26	6,3	400	600
40	26	6,3	600	600
41	26	7,3	0	85
42	26	7,3	200	85
43	26	7,3	400	85
44	26	7,3	600	85
45	26	7,3	0	600
46	26	7,3	200	600
47	26	7,3	400	600
48	26	7,3	600	600

Table A. 2. Full factorial design (cont.).

49	15	5,3	0	85
50	15	5,3	200	85
51	15	5,3	400	85
52	15	5,3	600	85
53	15	5,3	0	600
54	15	5,3	200	600
55	15	5,3	400	600
56	15	5,3	600	600
57	15	6,3	0	85
58	15	6,3	200	85
59	15	6,3	400	85
60	15	6,3	600	85
61	15	6,3	0	600
62	15	6,3	200	600
63	15	6,3	400	600
64	15	6,3	600	600
65	15	7,3	0	85
66	15	7,3	200	85
67	15	7,3	400	85
68	15	7,3	600	85
69	15	7,3	0	600
70	15	7,3	200	600
71	15	7,3	400	600
72	15	7,3	600	600

APPENDIX B. GOMPERTZ CURVES

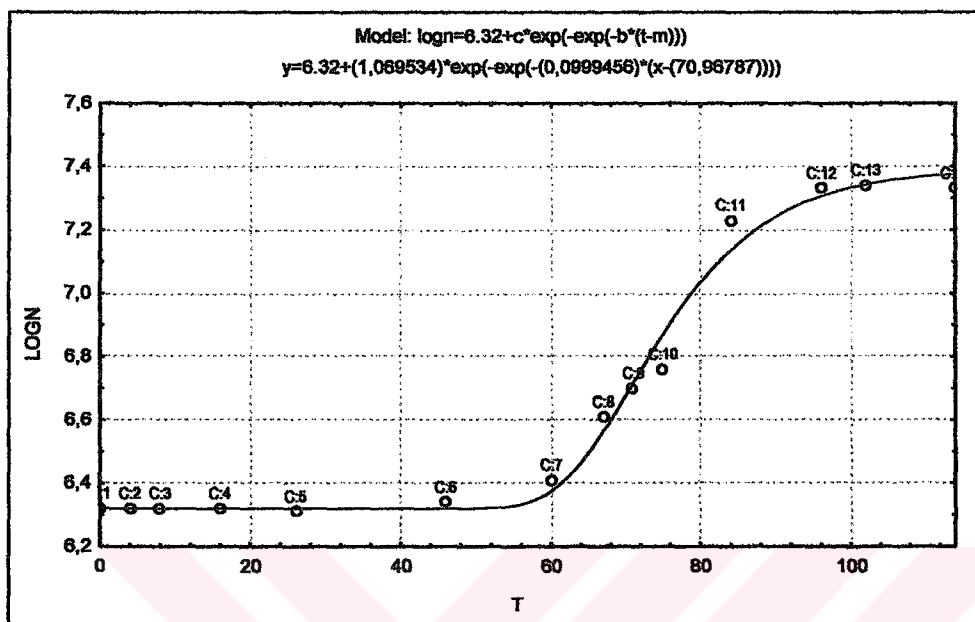


Figure B. 1. a. Growth curve of *B. cereus* at 8°C, 6.8 pH, 85 mM NaCl and without sodium lactate (T; time in h, LOGN; log(cfu/ml)).

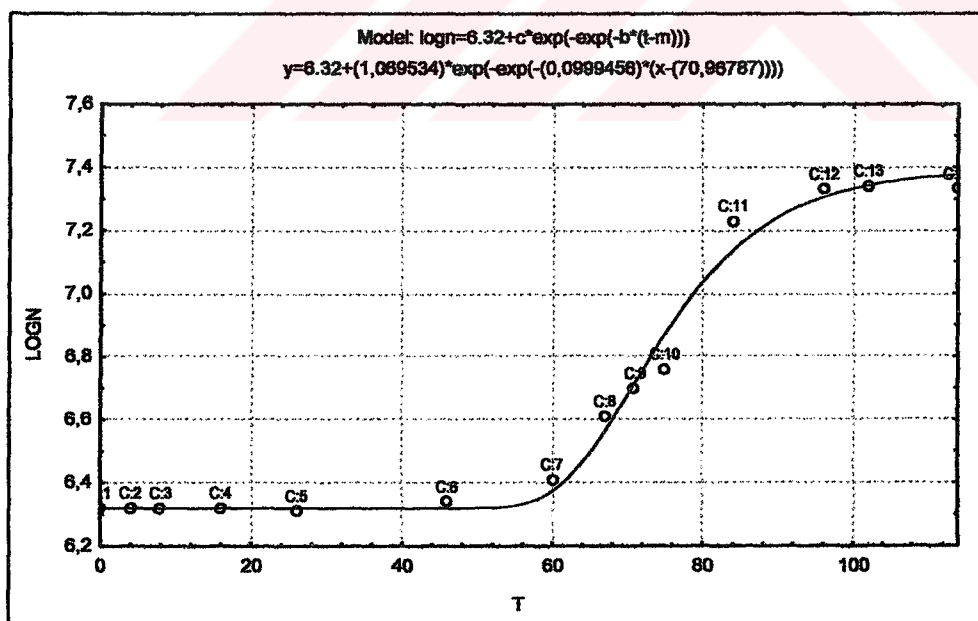


Figure B. 1. b. Growth curve of *B. cereus* at 8°C, 6.8 pH, 85 mM NaCl and without sodium lactate.

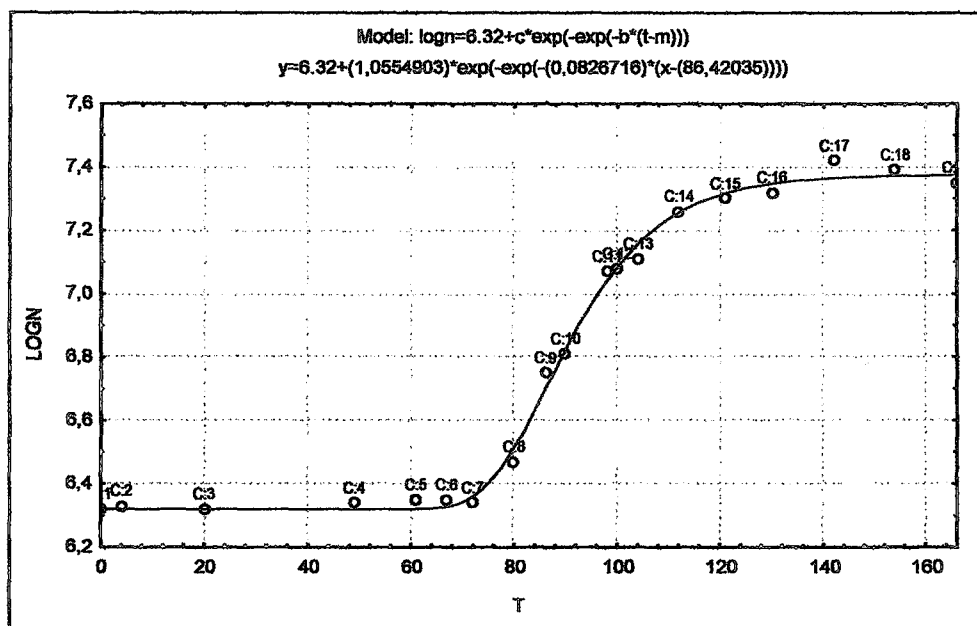


Figure B. 2. a. Growth curve of *B. cereus* at 8°C, 7.3 pH, 85 mM NaCl and without sodium lactate.

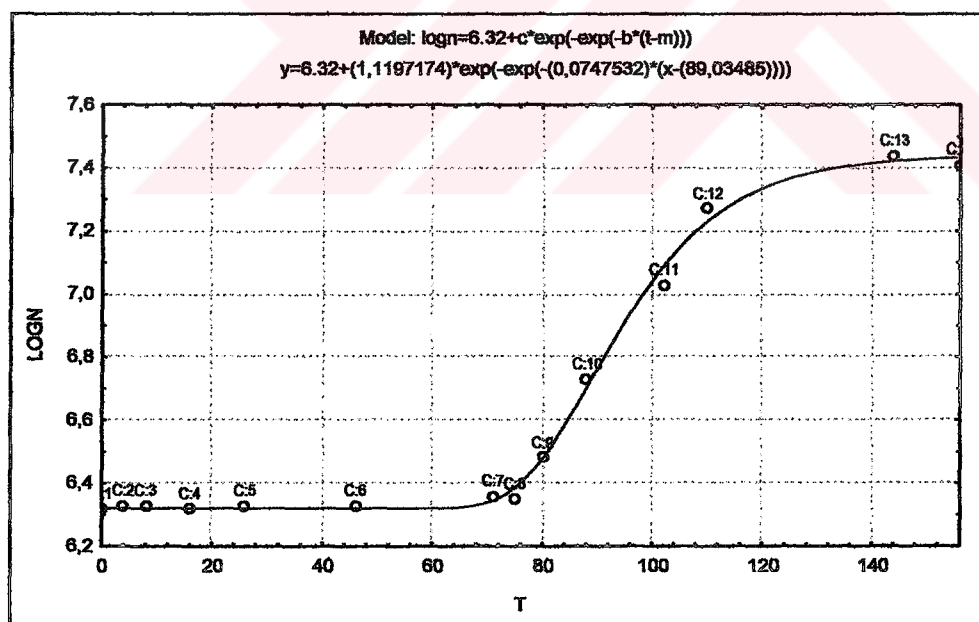


Figure B. 2. b. Growth curve of *B. cereus* at 8°C, 7.3 pH, 85 mM NaCl and without sodium lactate.

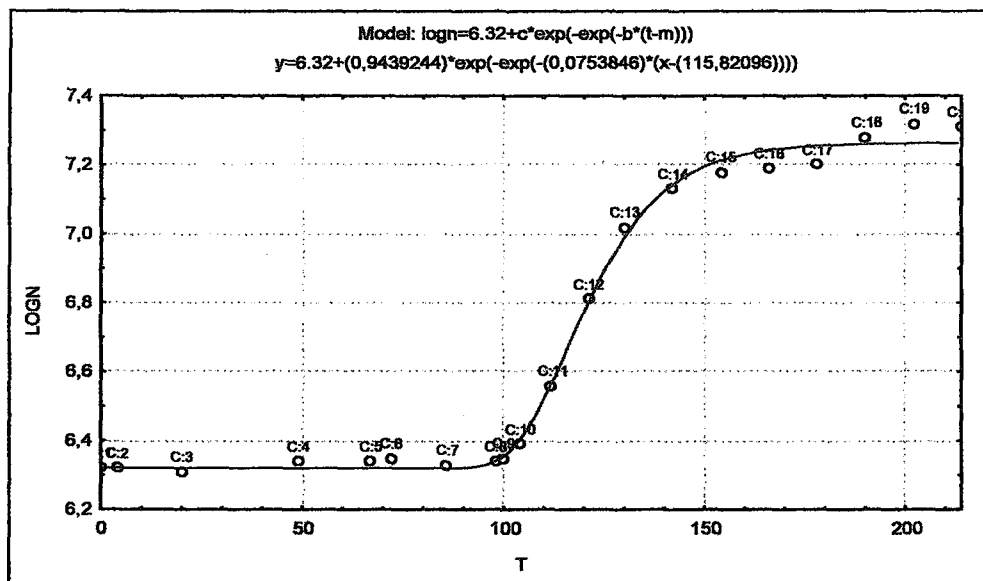


Figure B. 3. a. Growth curve of *B. cereus* at 8°C, 7.3 pH, 85 mM NaCl and 200 mM sodium lactate.

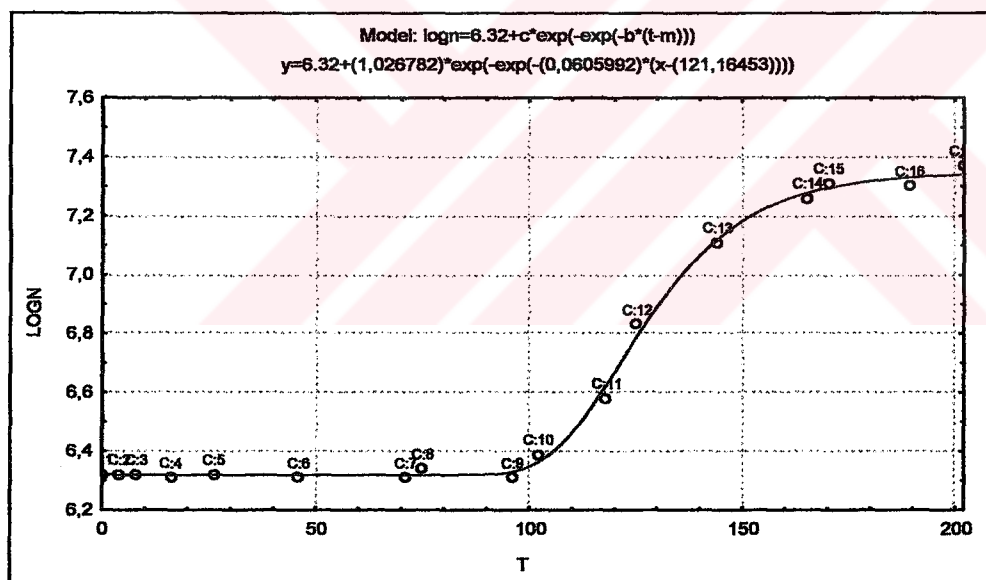


Figure B. 3. b. Growth curve of *B. cereus* at 8°C, 7.3 pH, 85 mM NaCl and 200 mM sodium lactate.

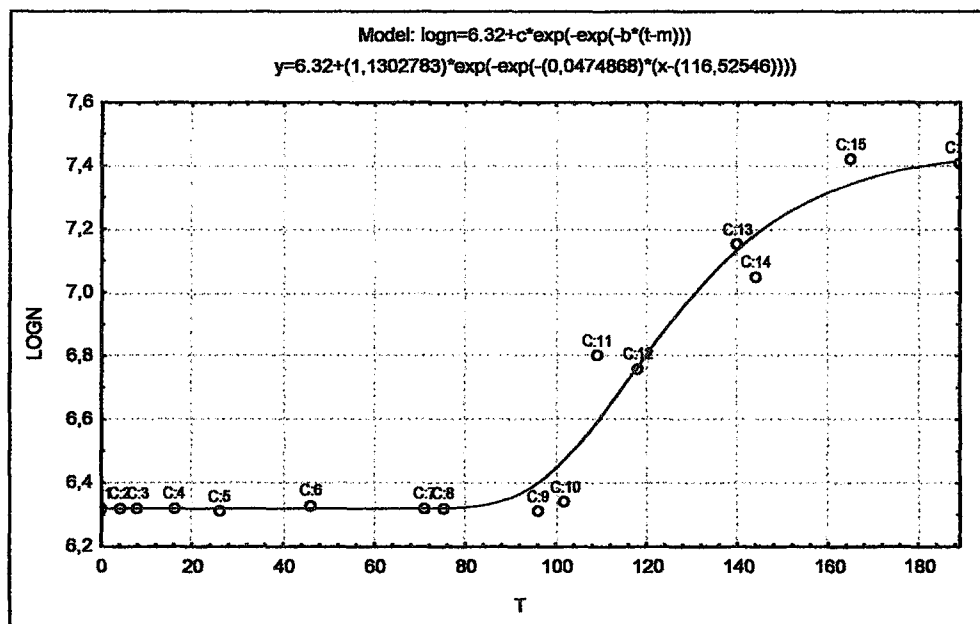


Figure B. 4. a. Growth curve of *B. cereus* at 8°C, 7.3 pH, 342 mM NaCl and without sodium lactate.

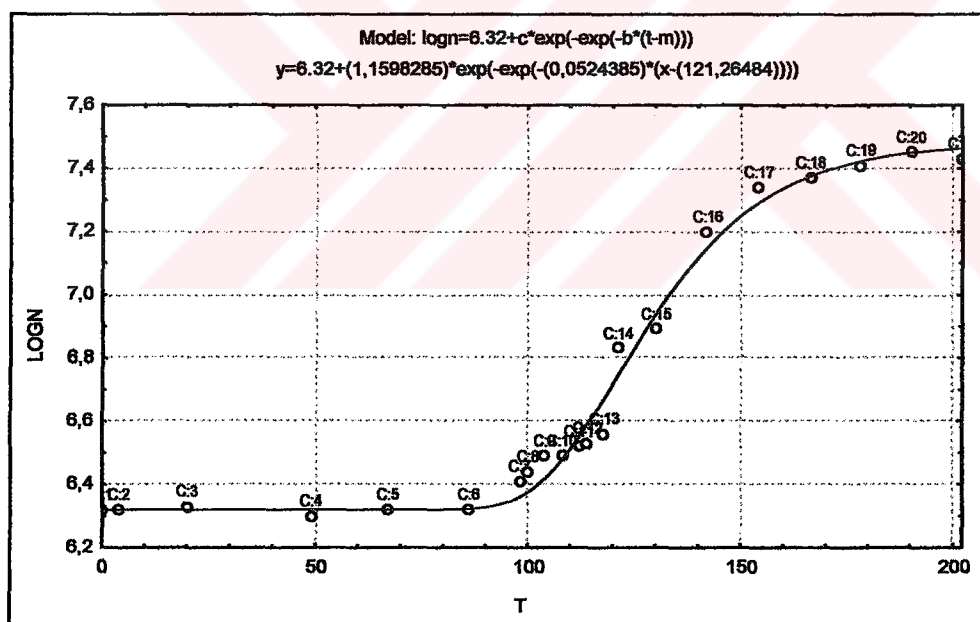


Figure B. 4. b. Growth curve of *B. cereus* at 8°C, 7.3 pH, 342 mM NaCl and without sodium lactate.

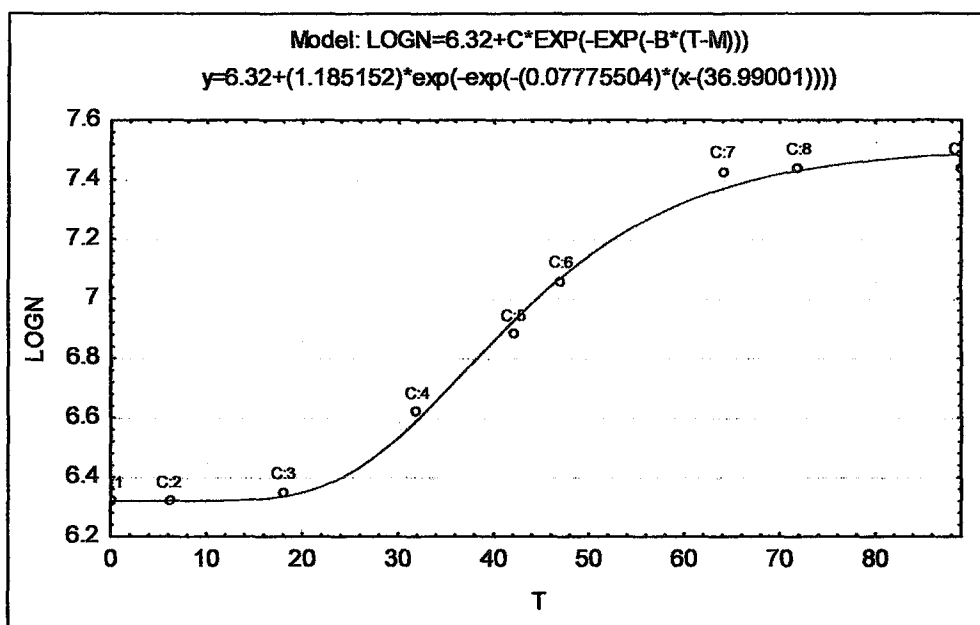


Figure B. 5. a. Growth curve of *B. cereus* at 15°C, 5.3 pH, 85 mM NaCl and without sodium lactate.

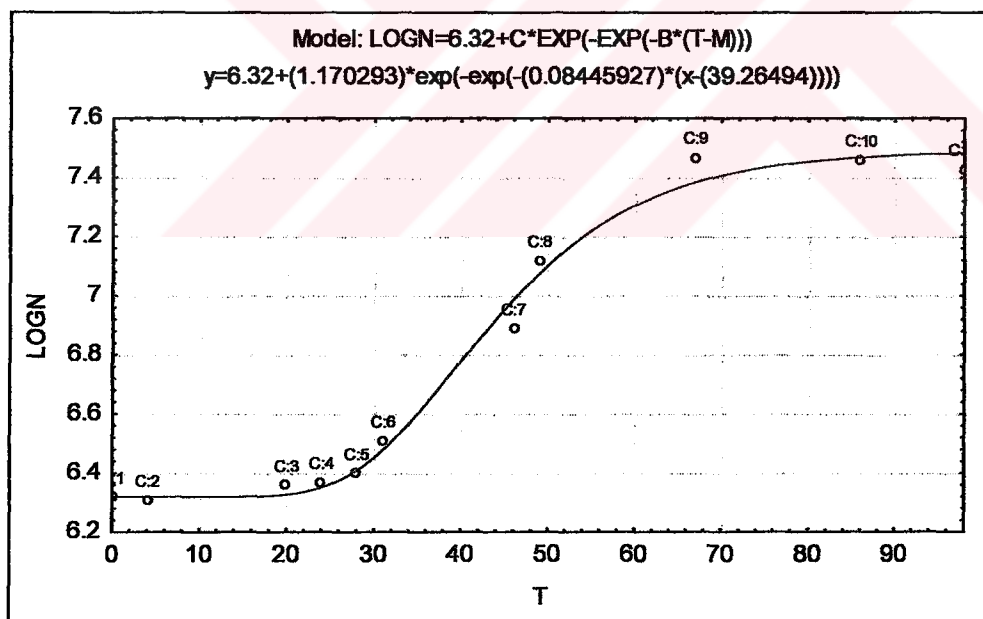


Figure B. 5. b. Growth curve of *B. cereus* at 15°C, 5.3 pH, 85 mM NaCl and without sodium lactate.

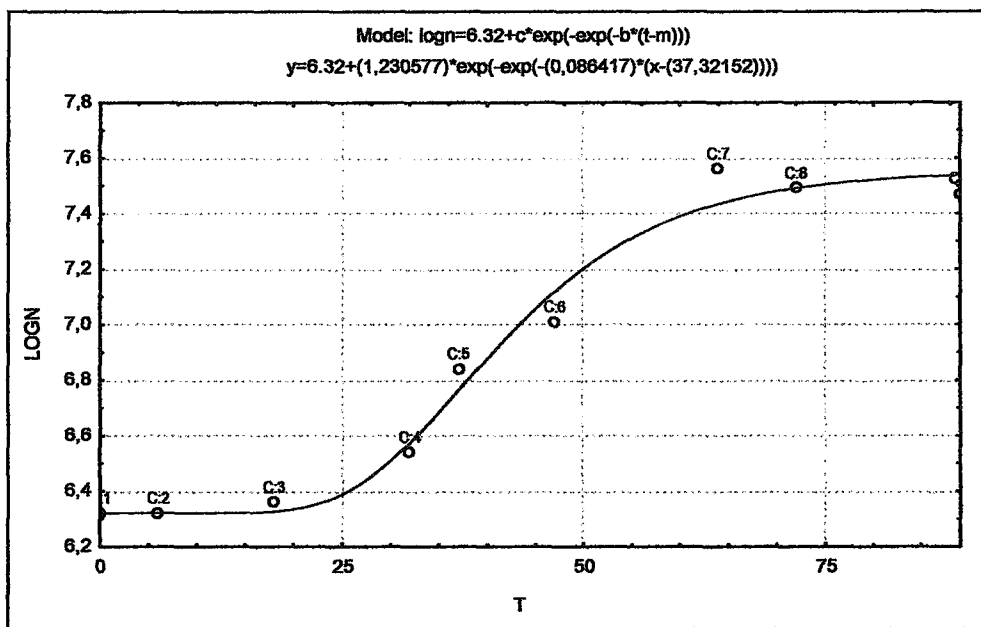


Figure B. 6. a. Growth curve of *B. cereus* at 15°C, 5.8 pH, 85 mM NaCl and without sodium lactate.

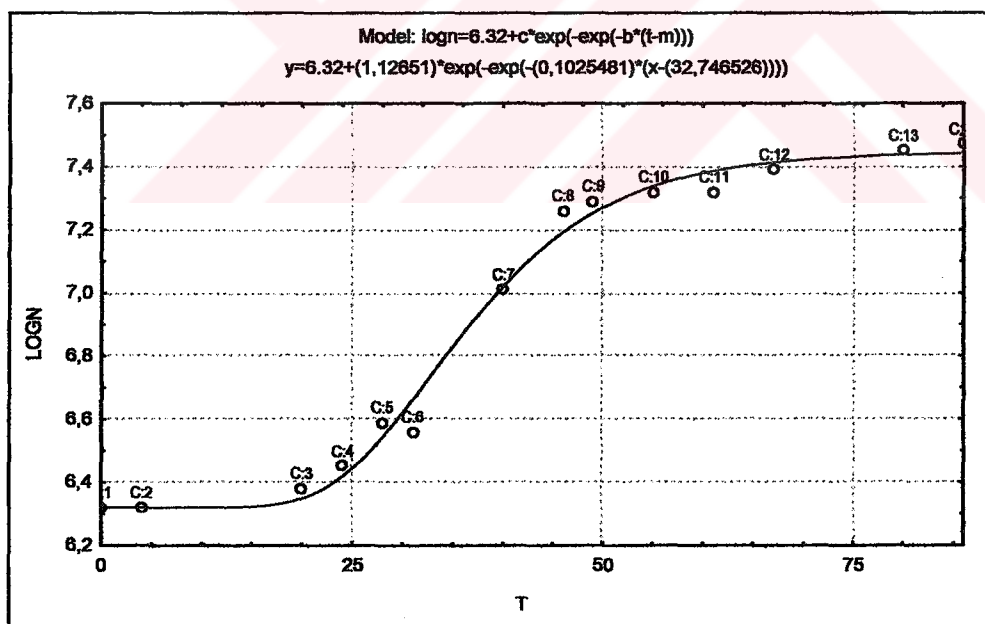


Figure B. 6. b. Growth curve of *B. cereus* at 15°C, 5.8 pH, 85 mM NaCl and without sodium lactate.

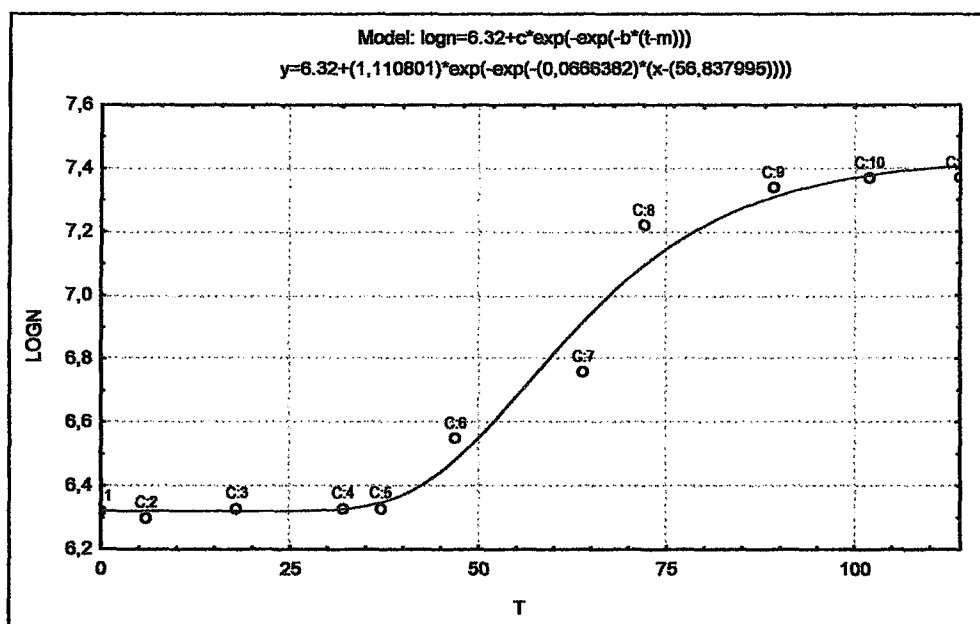


Figure B. 7. a. Growth curve of *B. cereus* at 15°C, 5.8 pH, 85 mM NaCl and 200 mM sodium lactate.

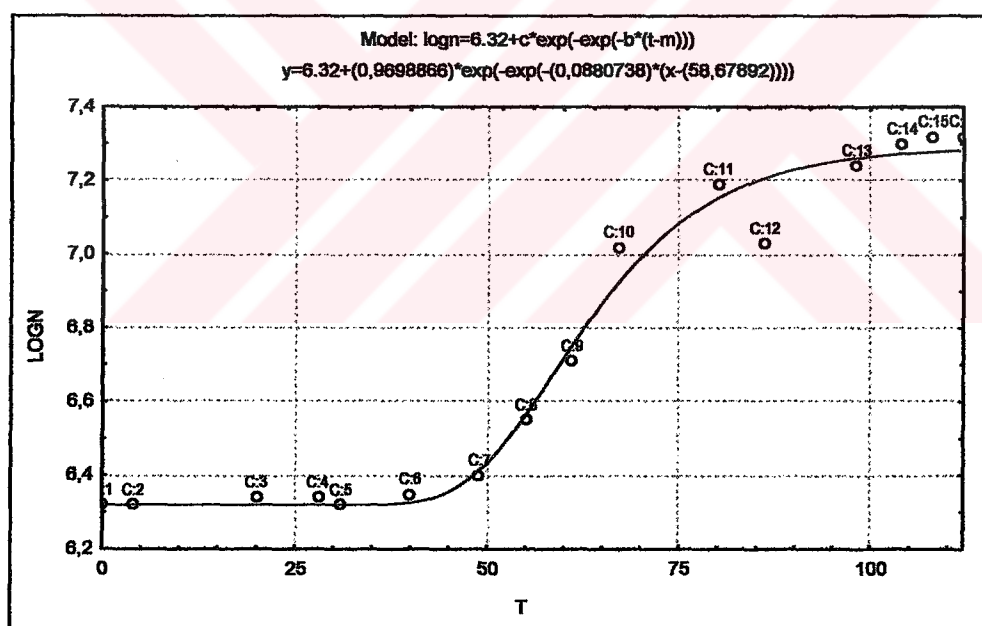


Figure B. 7. b. Growth curve of *B. cereus* at 15°C, 5.8 pH, 85 mM NaCl and 200 mM sodium lactate.

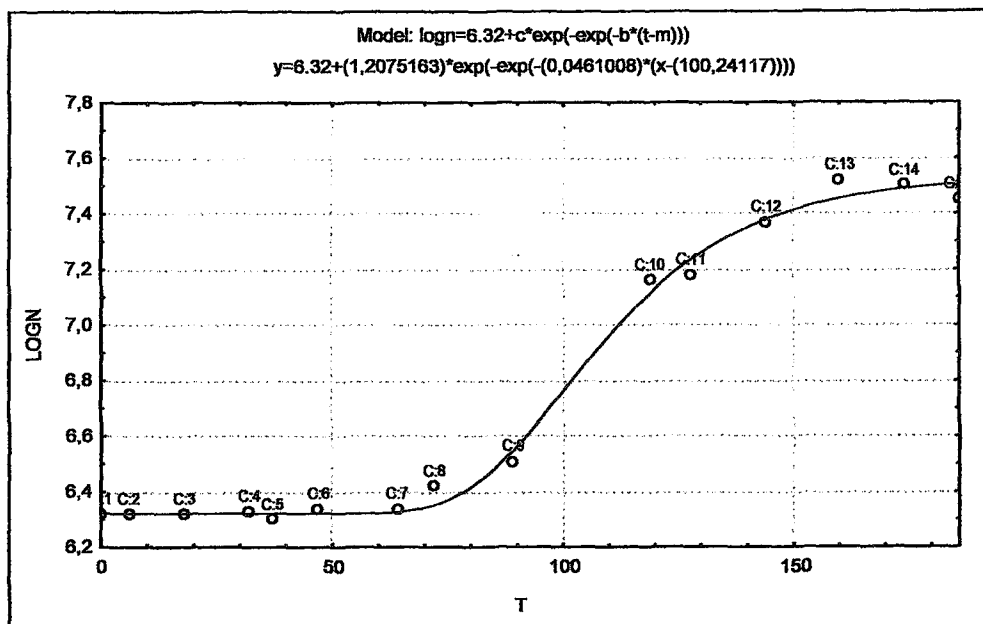


Figure B. 8. a. Growth curve of *B. cereus* at 15°C, 5.8 pH, 600 mM NaCl and 200 mM sodium lactate.

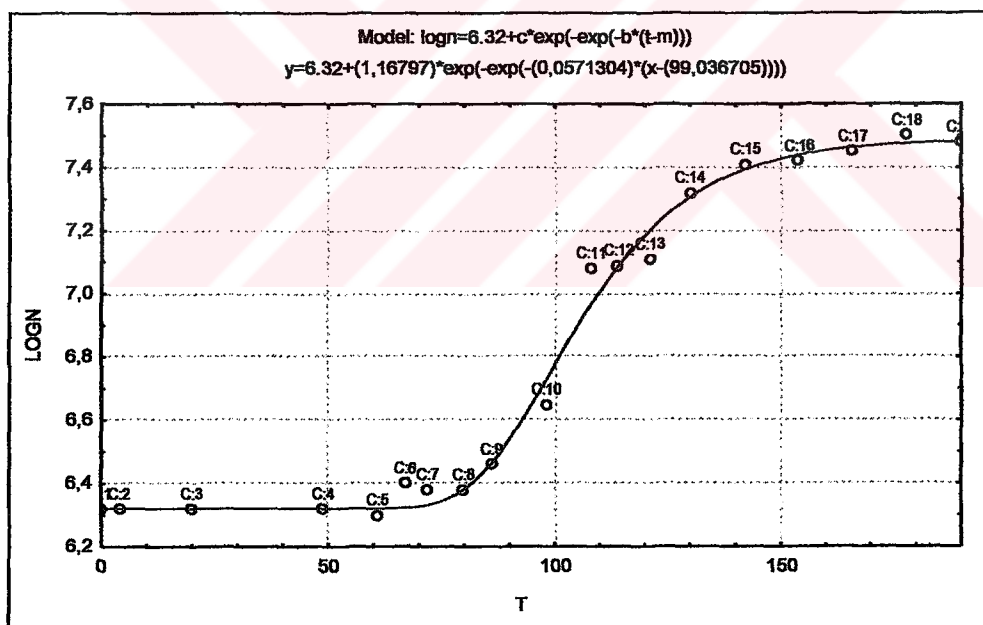


Figure B. 8. b. Growth curve of *B. cereus* at 15°C, 5.8 pH, 600 mM NaCl and 200 mM sodium lactate.

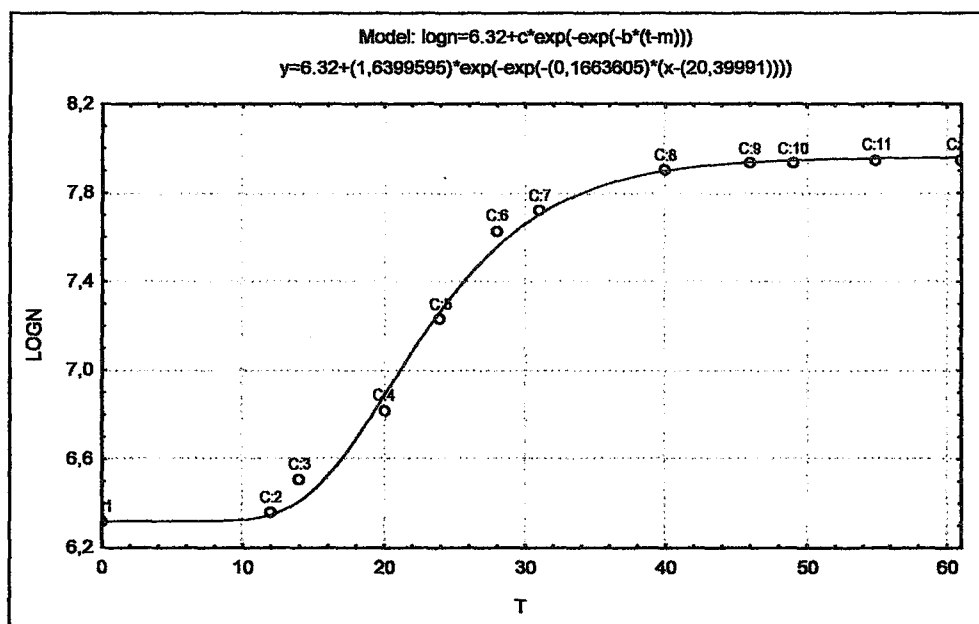


Figure B. 9. a. Growth curve of *B. cereus* at 15°C, 6.3 pH, 85 mM NaCl and without sodium lactate.

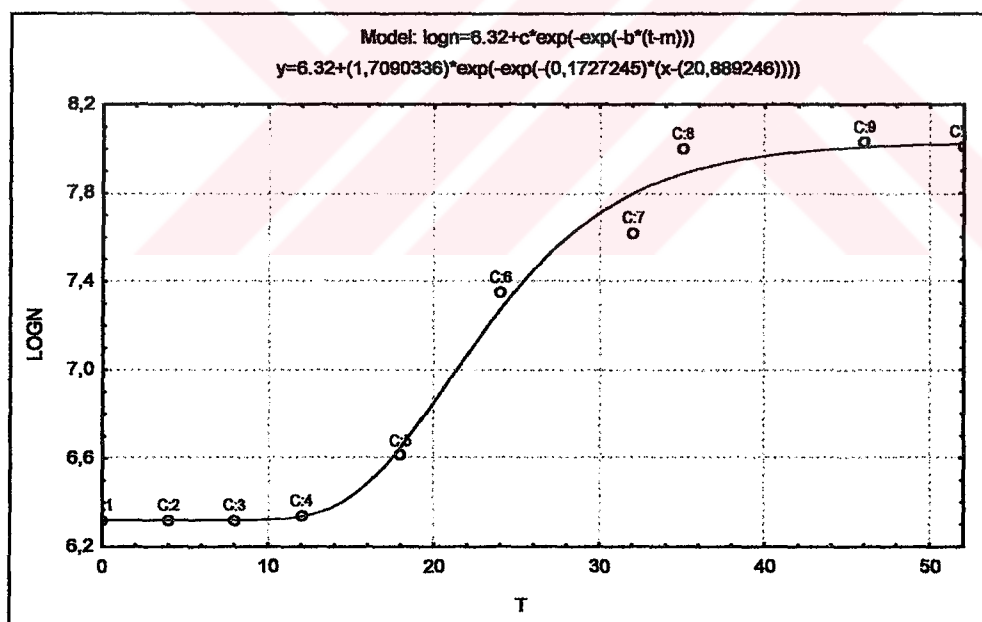


Figure B. 9. b. Growth curve of *B. cereus* at 15°C, 6.3 pH, 85 mM NaCl and without sodium lactate.

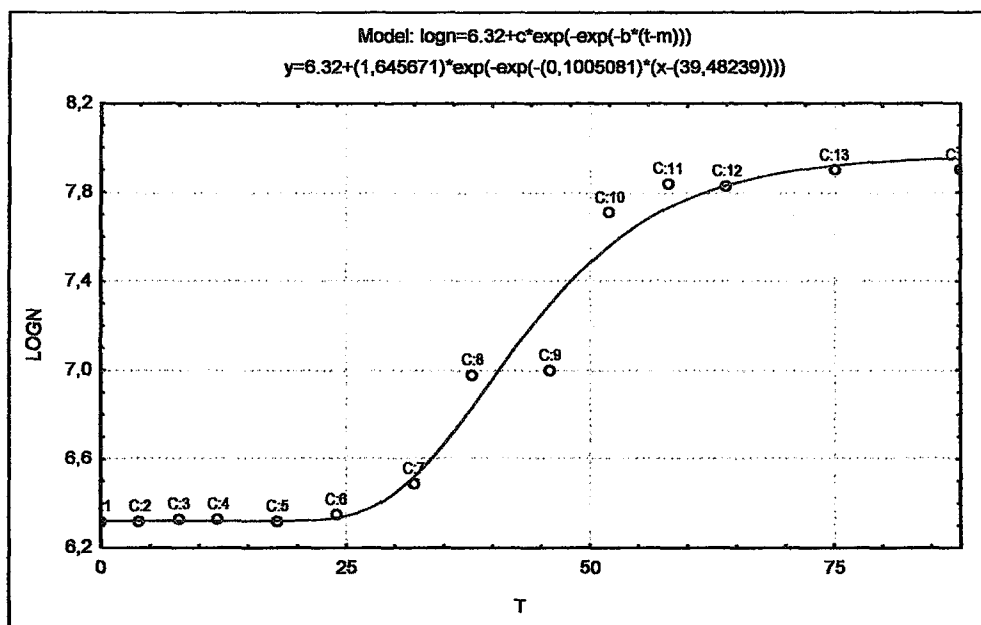


Figure B. 10. a. Growth curve of *B. cereus* at 15°C, 6.3 pH, 85 mM NaCl and 200 mM sodium lactate.

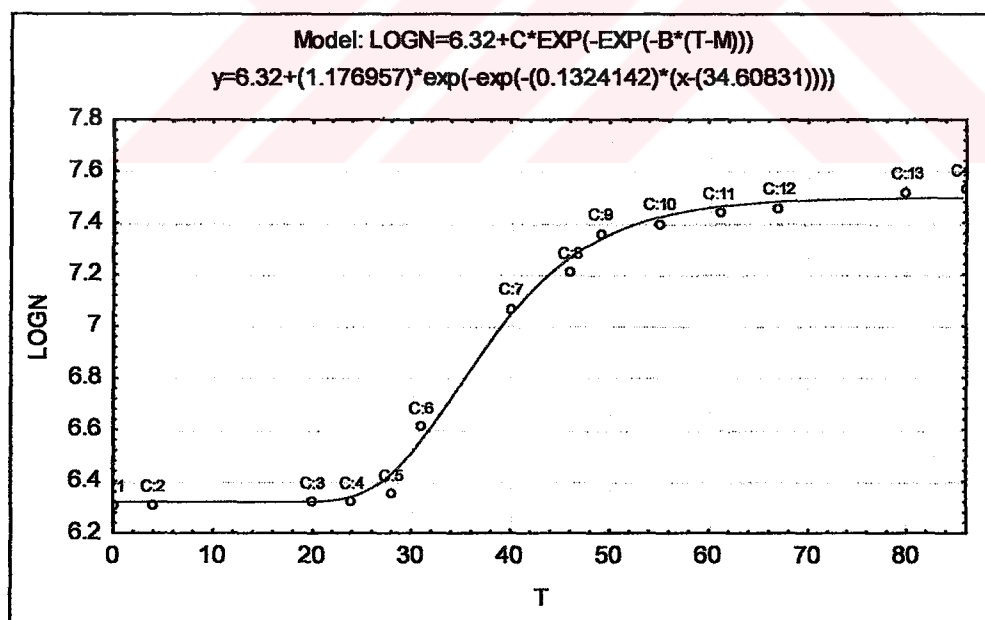


Figure B. 10. b. Growth curve of *B. cereus* at 15°C, 6.3 pH, 85 mM NaCl and 200 mM sodium lactate.

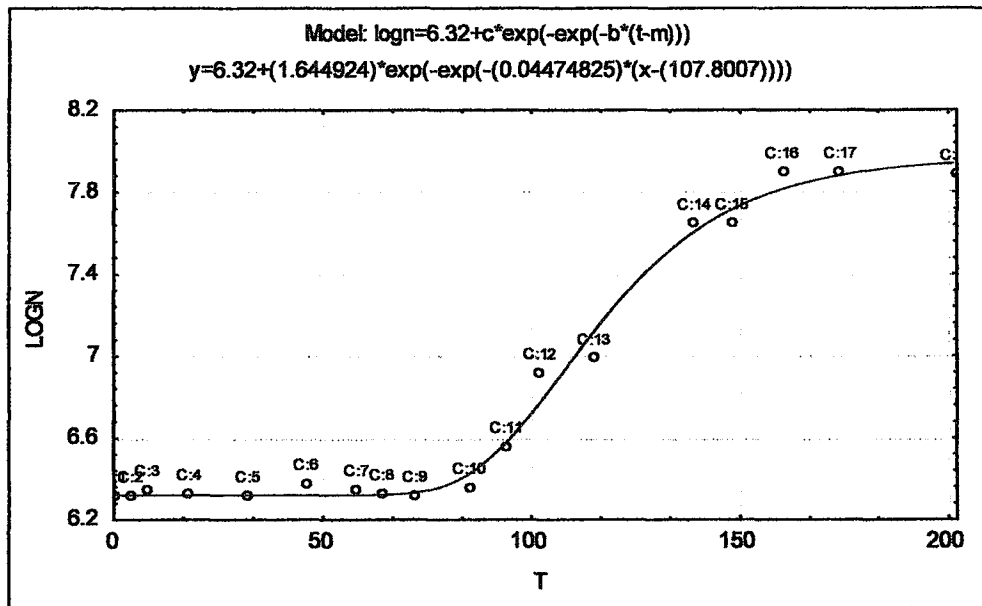


Figure B. 11. Growth curve of *B. cereus* at 15°C, 6.3 pH, 85 mM NaCl and 400 mM sodium lactate.

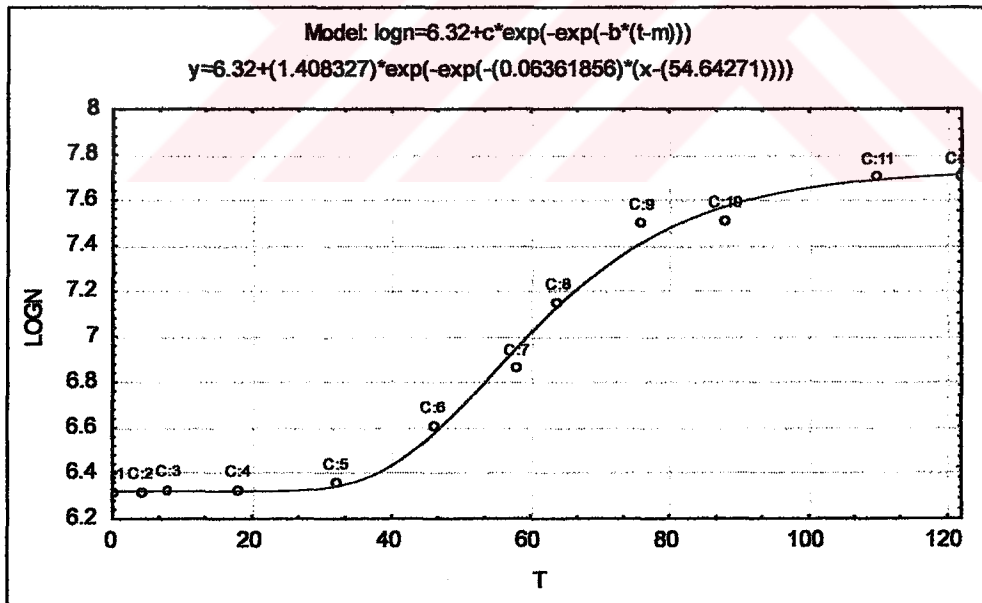


Figure B. 12. Growth curve of *B. cereus* at 15°C, 6.3 pH, 600 mM NaCl and without sodium lactate.

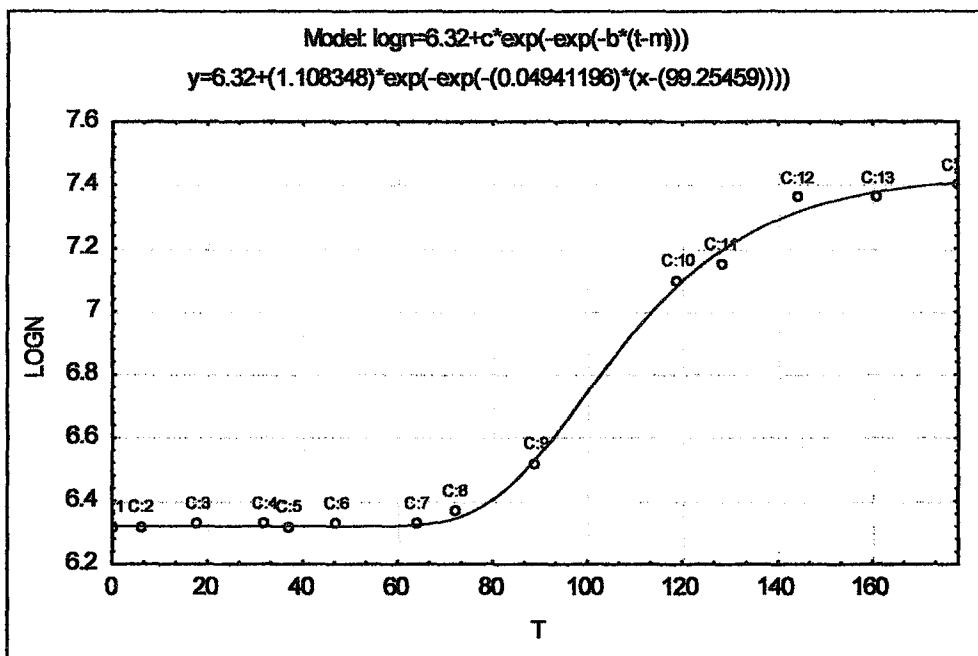


Figure B. 13. Growth curve of *B. cereus* at 15°C, 6.3 pH, 600 mM NaCl and 200 mM sodium lactate.

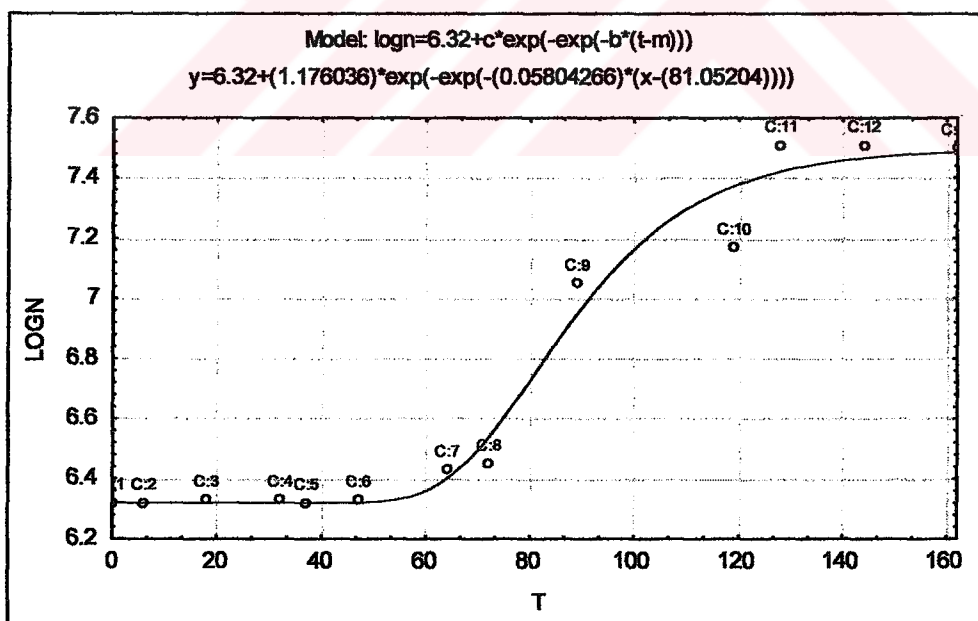


Figure B. 14. Growth curve of *B. cereus* at 15°C, 6.3 pH, 342 mM NaCl and 200 mM sodium lactate.

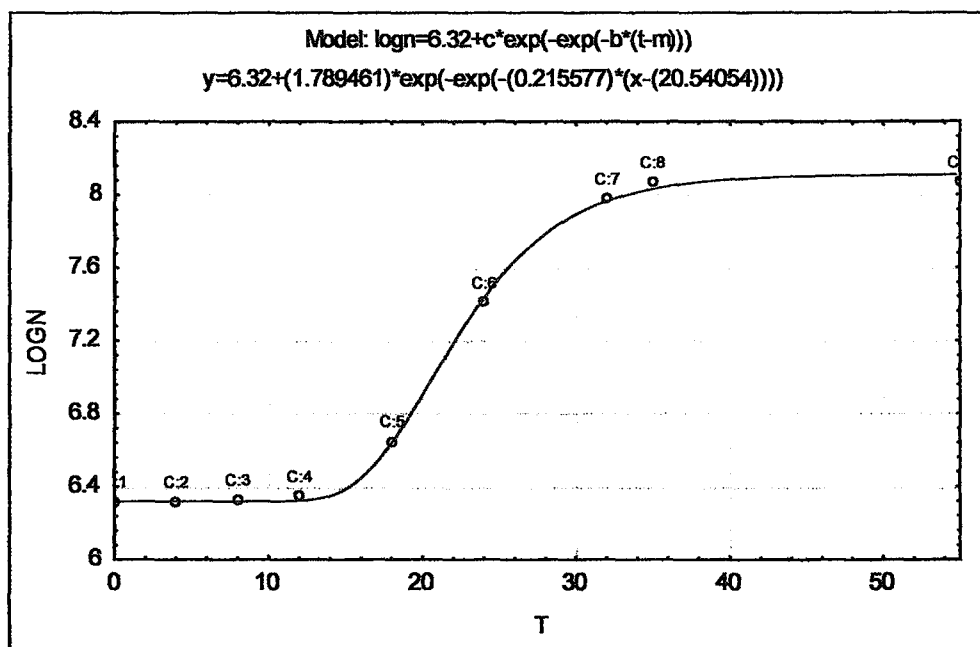


Figure B. 15. Growth curve of *B. cereus* at 15°C, 6.8 pH, 85 mM NaCl and without sodium lactate.

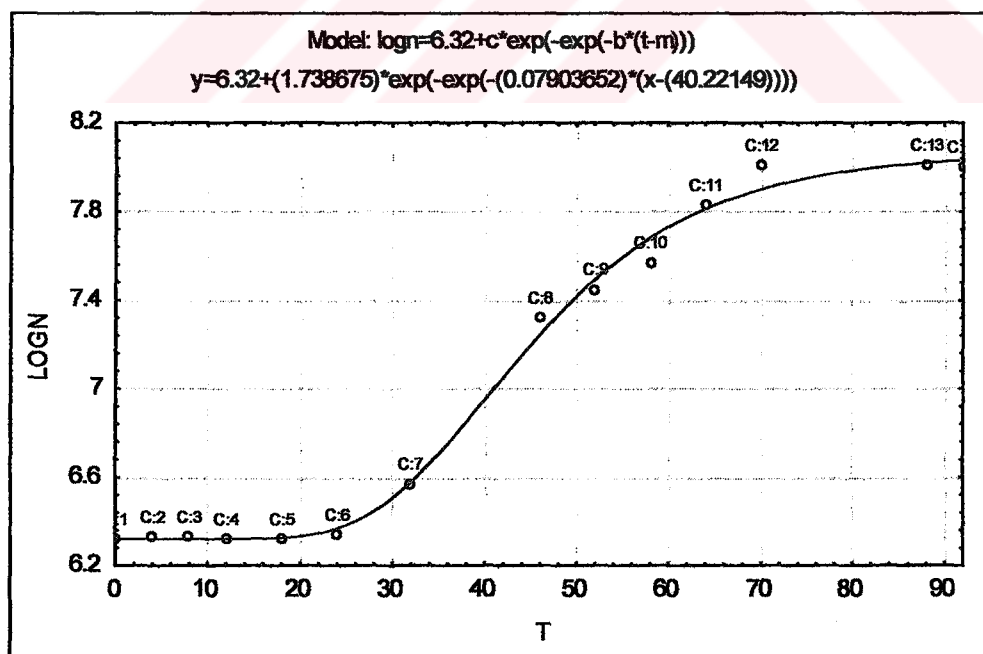


Figure B. 16. Growth curve of *B. cereus* at 15°C, 6.8 pH, 85 mM NaCl and 200 mM sodium lactate.

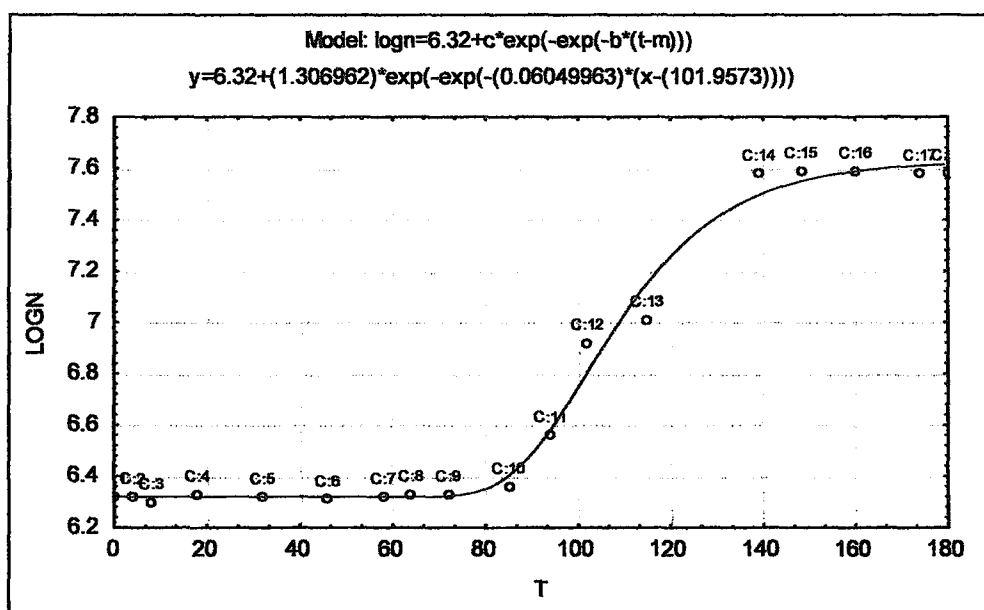


Figure B. 17. Growth curve of *B. cereus* at 15°C, 6.8 pH, 85 mM NaCl and 400 mM sodium lactate.

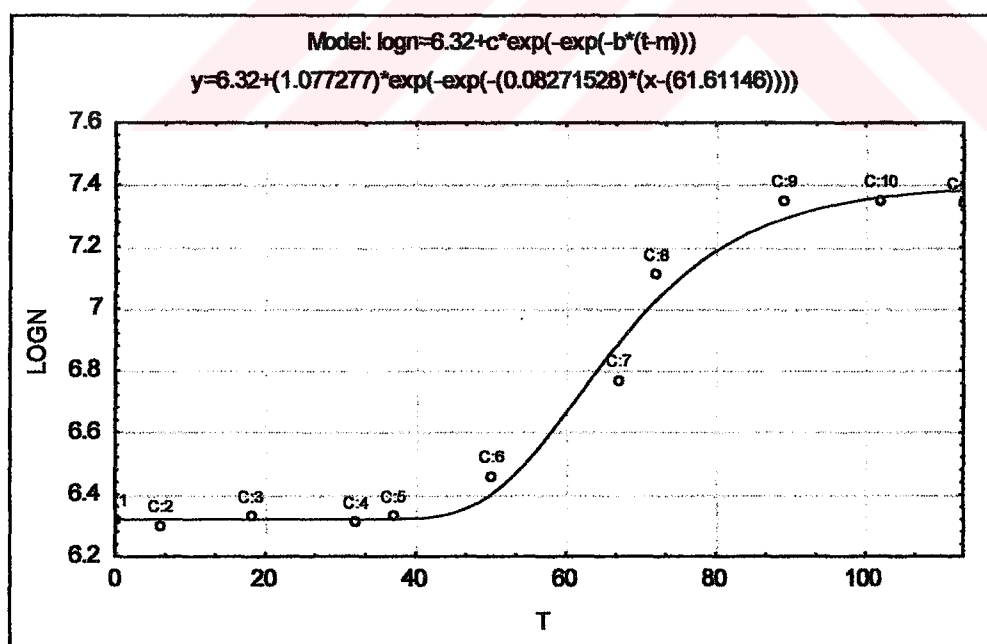


Figure B. 18. Growth curve of *B. cereus* at 15°C, 6.8 pH, 342 mM NaCl and 200 mM sodium lactate.

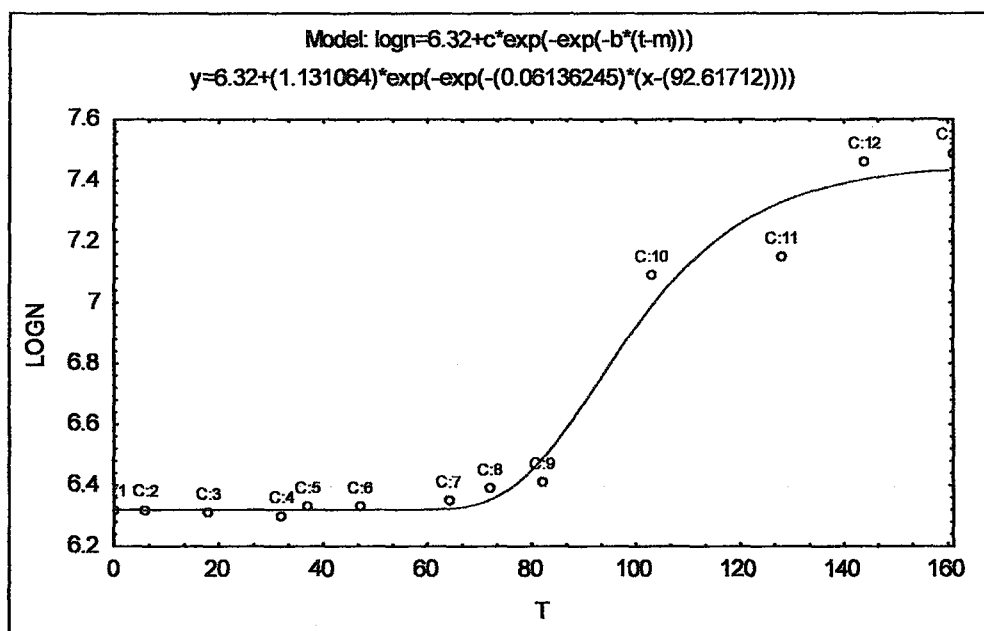


Figure B. 19. Growth curve of *B. cereus* at 15°C, 6.8 pH, 600 mM NaCl and 200 mM sodium lactate.

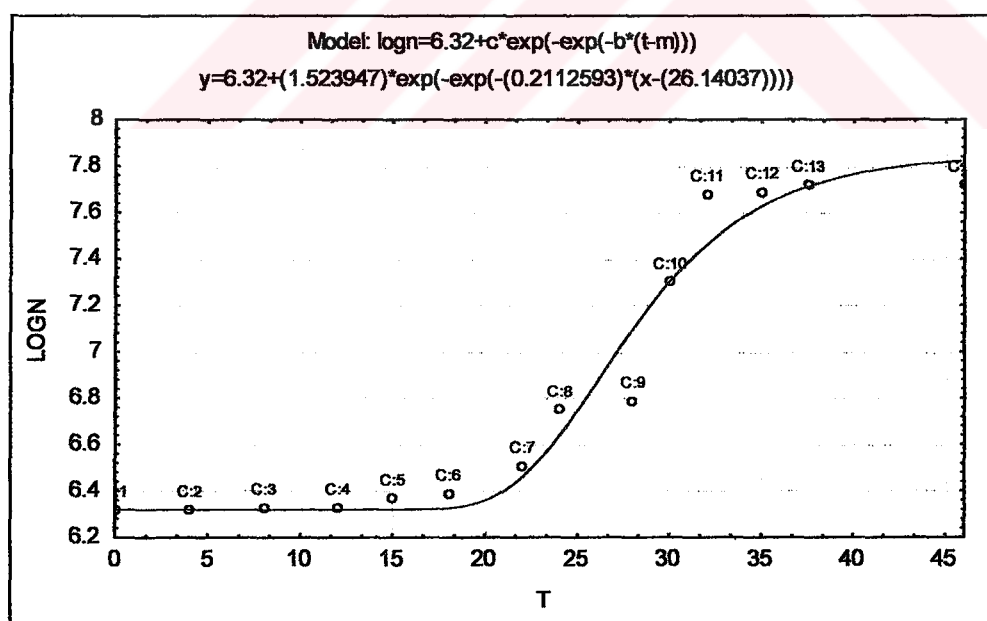


Figure B. 20. Growth curve of *B. cereus* at 15°C, 7.3 pH, 85 mM NaCl and without sodium lactate.

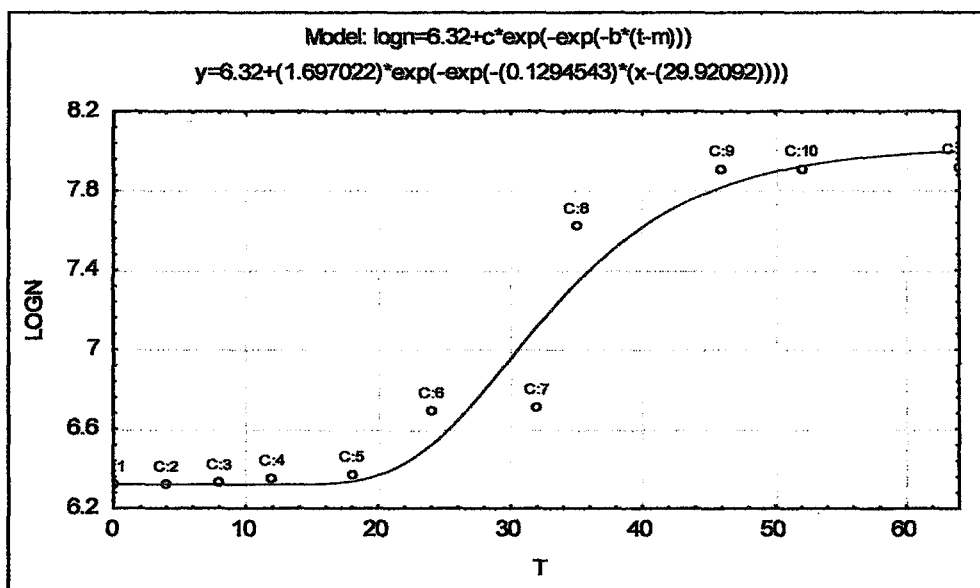


Figure B. 21. Growth curve of *B. cereus* at 15°C, 7.3 pH, 85 mM NaCl and 200 mM sodium acetate.

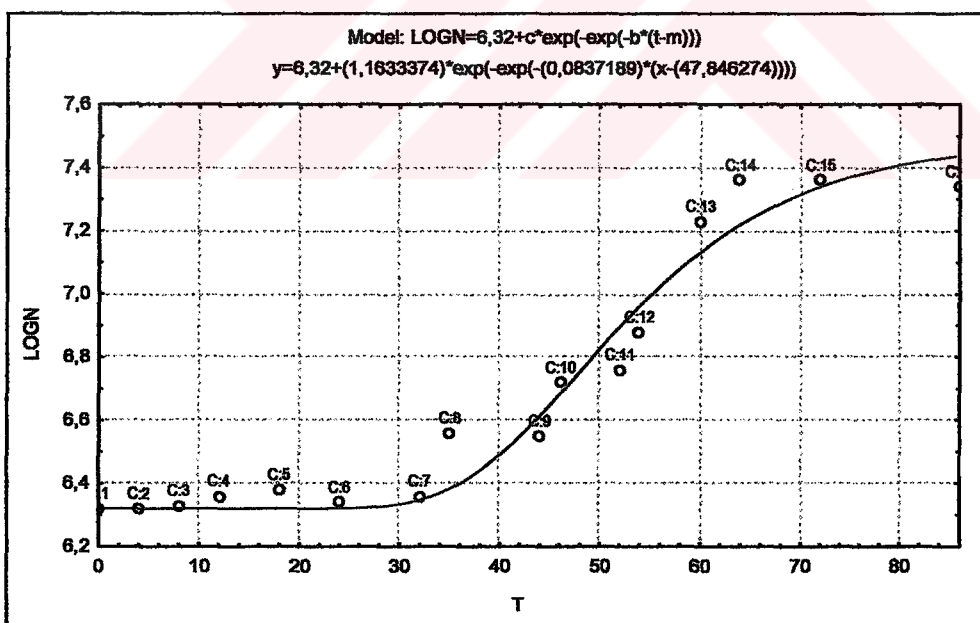


Figure B. 22. a. Growth curve of *B. cereus* at 15°C, 7.3 pH, 85 mM NaCl and 400 mM sodium lactate.

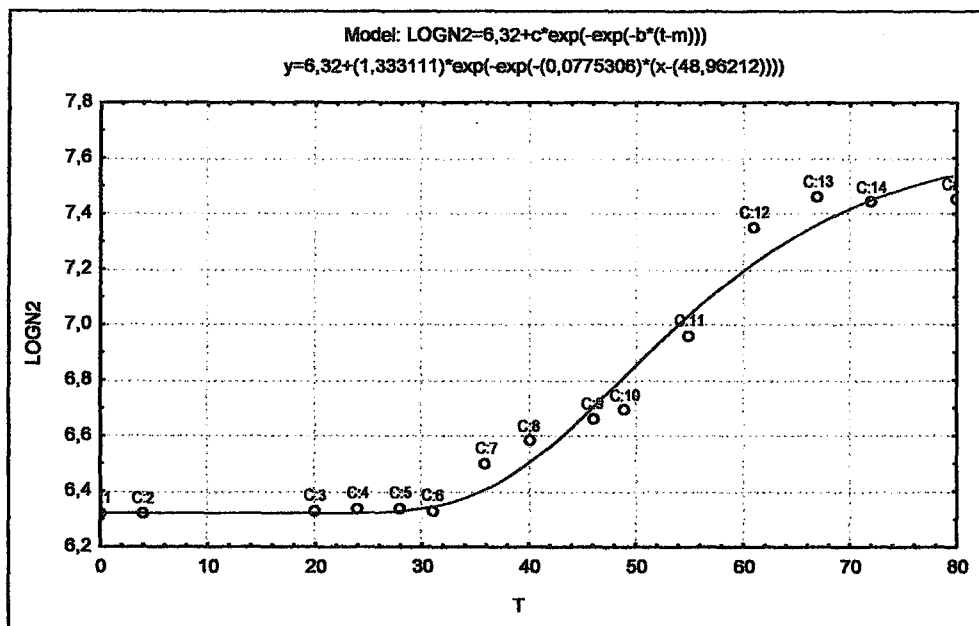


Figure B. 22. b. Growth curve of *B. cereus* at 15°C, 7.3 pH, 85 mM NaCl and 400 mM sodium lactate.

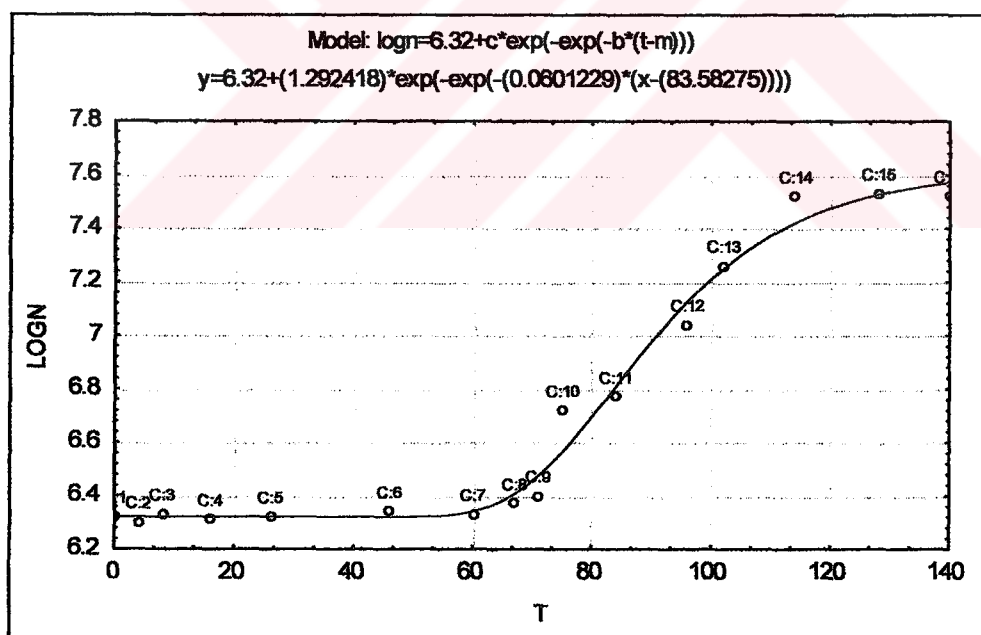


Figure B. 23. Growth curve of *B. cereus* at 15°C, 7.3 pH, 85 mM NaCl and 600 mM sodium lactate.

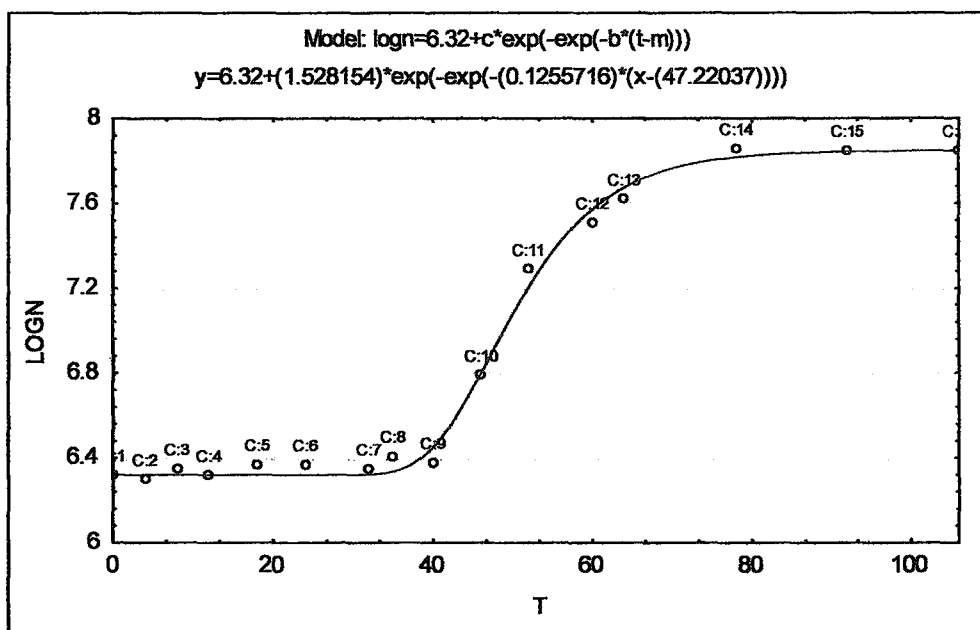


Figure B. 24. Growth curve of *B. cereus* at 15°C, 7.3 pH, 600 mM NaCl and without sodium lactate.

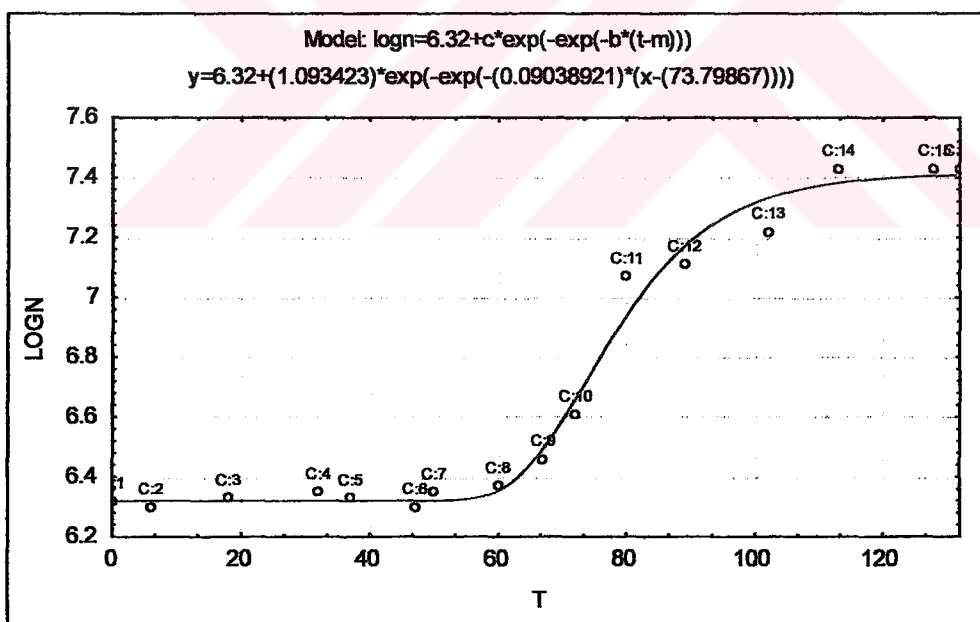


Figure B. 25. Growth curve of *B. cereus* at 15°C, 7.3 pH, 600 mM NaCl and 200 mM sodium lactate.

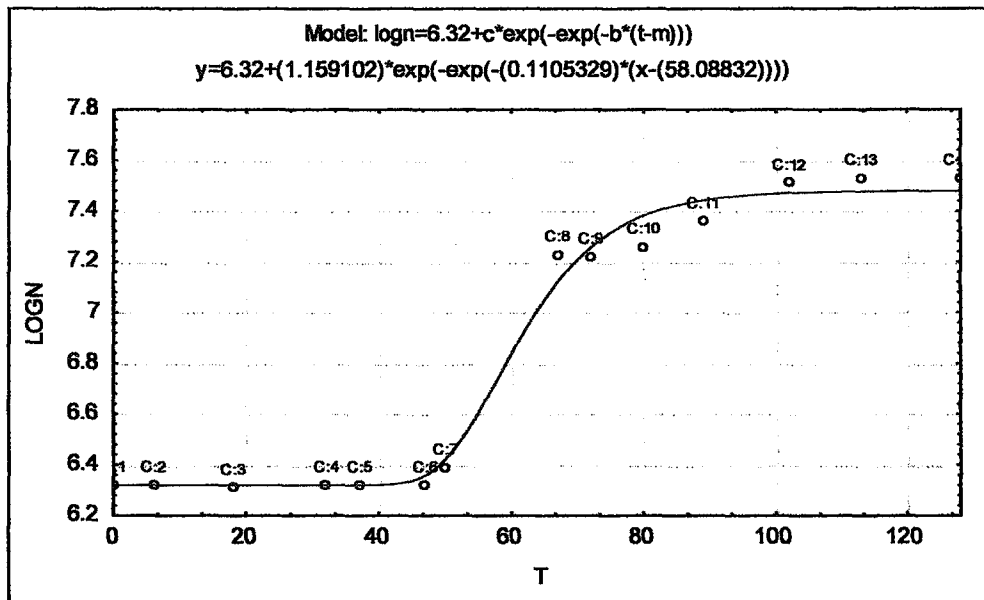


Figure B. 26. Growth curve of *B. cereus* at 15°C, 7.3 pH, 342 mM NaCl and 200 mM sodium lactate.

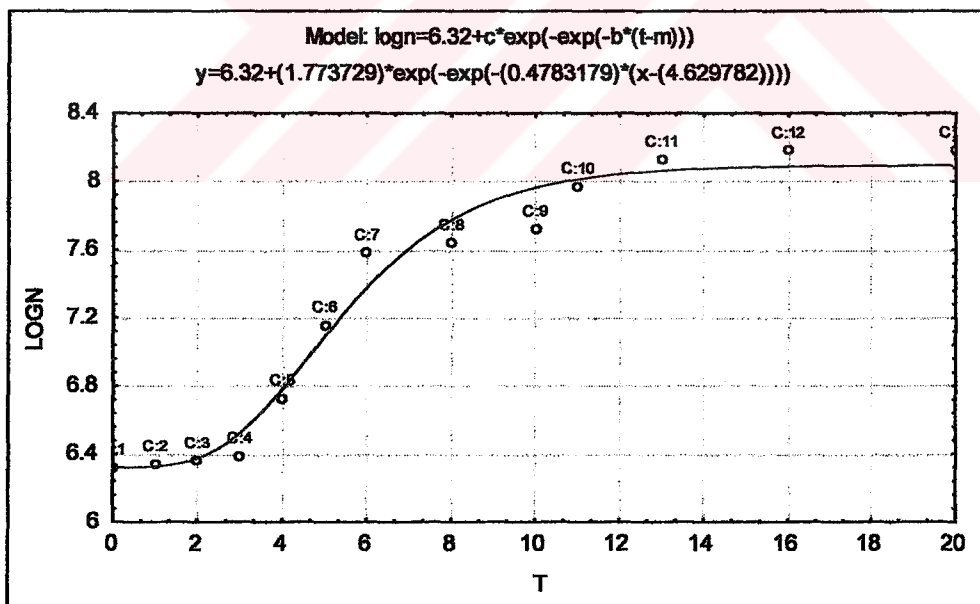


Figure B. 27. Growth curve of *B. cereus* at 26°C, 5.3 pH, 85 mM NaCl and without sodium lactate.

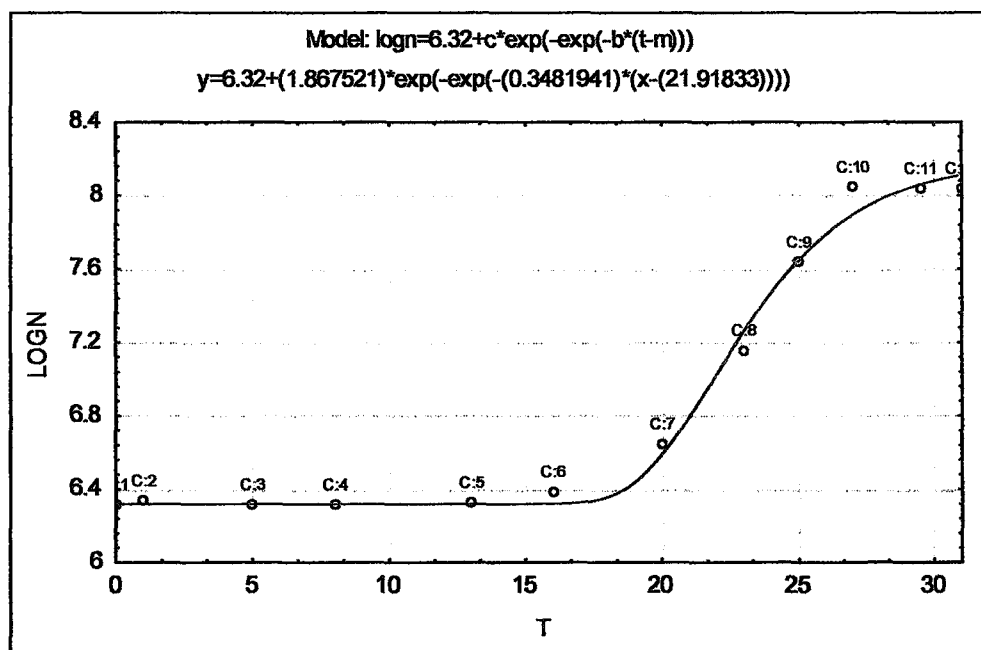


Figure B. 28. Growth curve of *B. cereus* at 26°C, 5.3 pH, 600 mM NaCl and without sodium lactate.

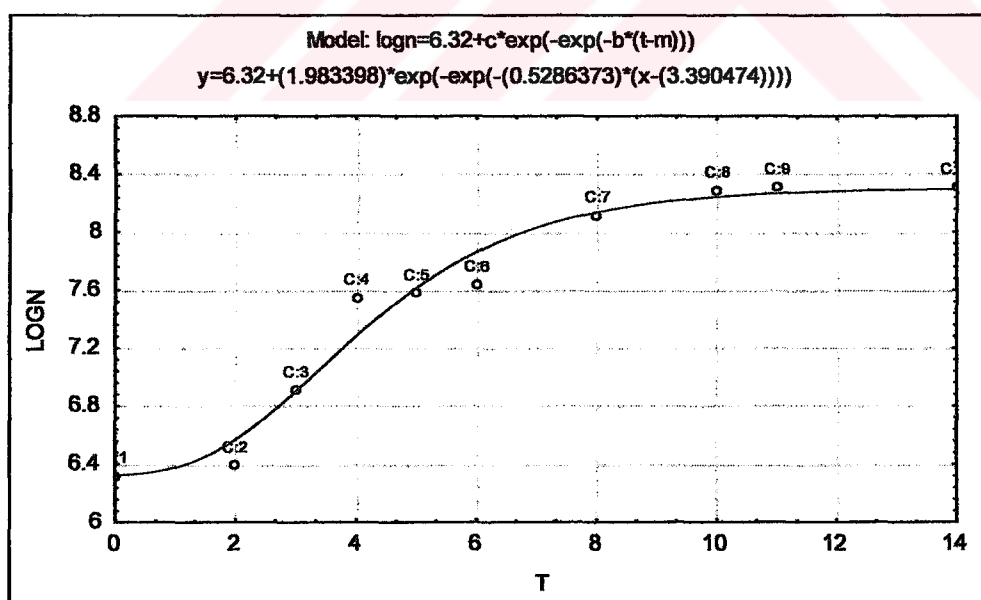


Figure B. 29. Growth curve of *B. cereus* at 26°C, 5.8 pH, 85 mM NaCl and without sodium lactate.

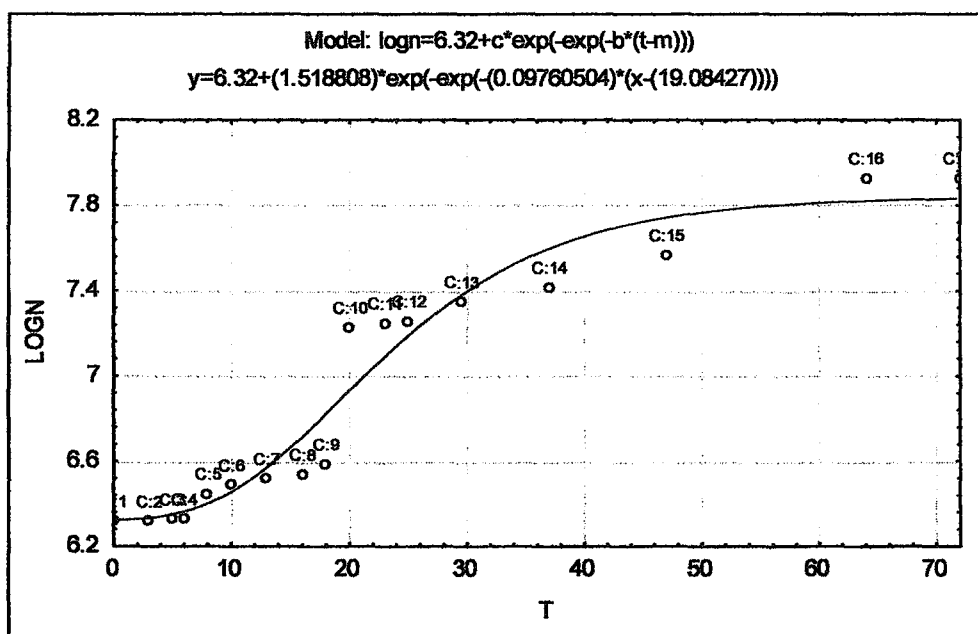


Figure B. 30. Growth curve of *B. cereus* at 26°C, 5.8 pH, 85 mM NaCl and 200 mM sodium lactate.

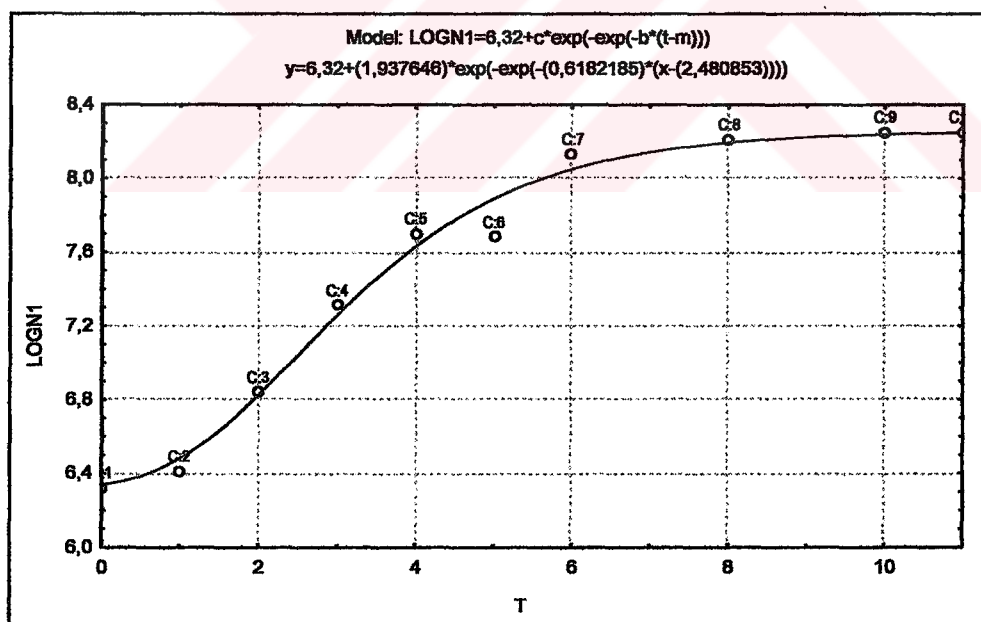


Figure B. 31. a. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and without sodium lactate.

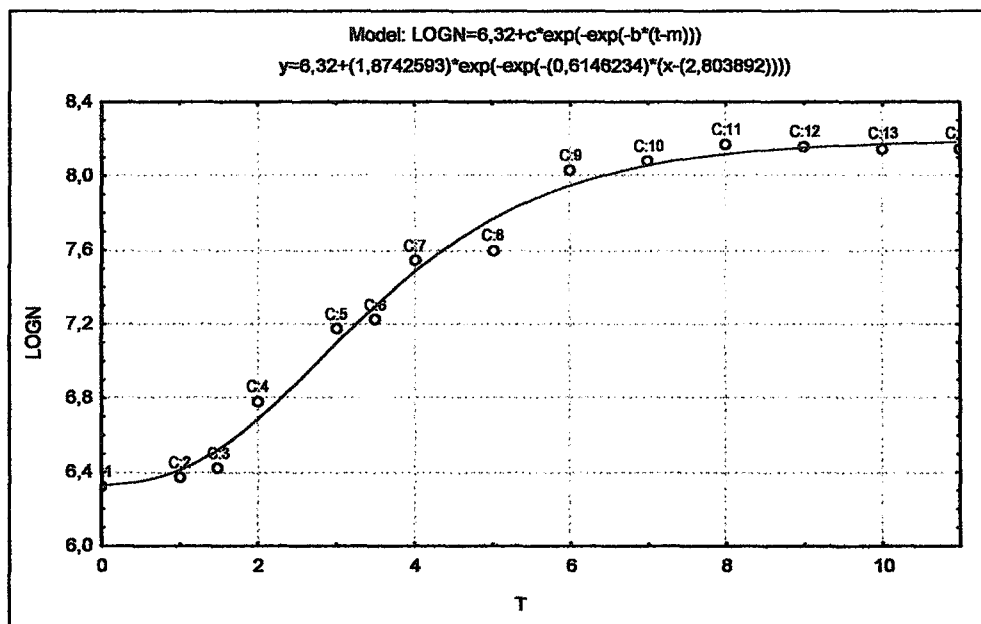


Figure B. 31. b. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and without sodium lactate.

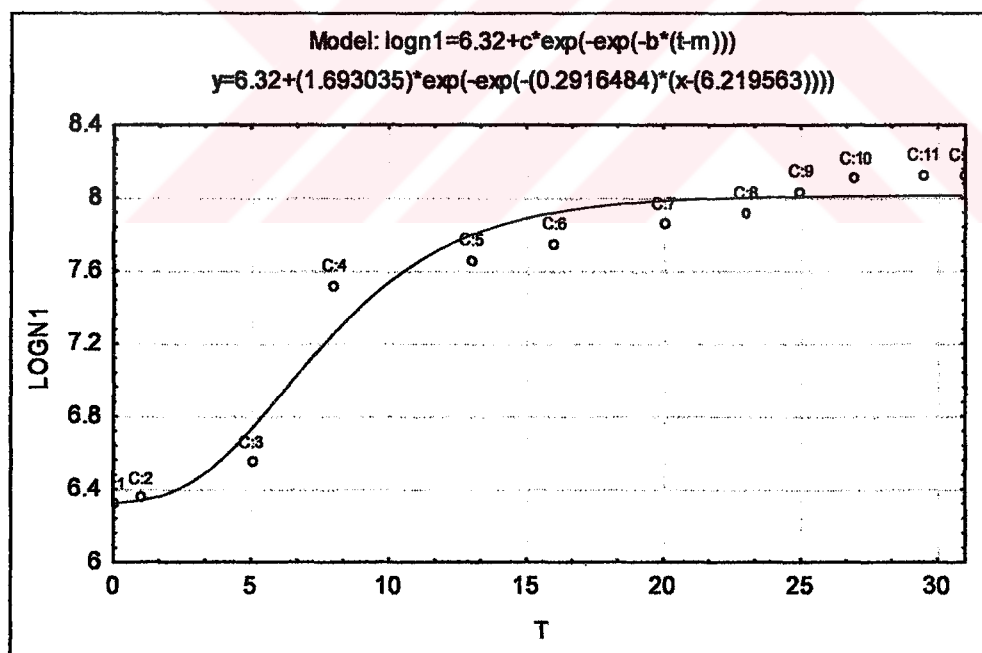


Figure B. 32. a. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and 200 mM sodium lactate.

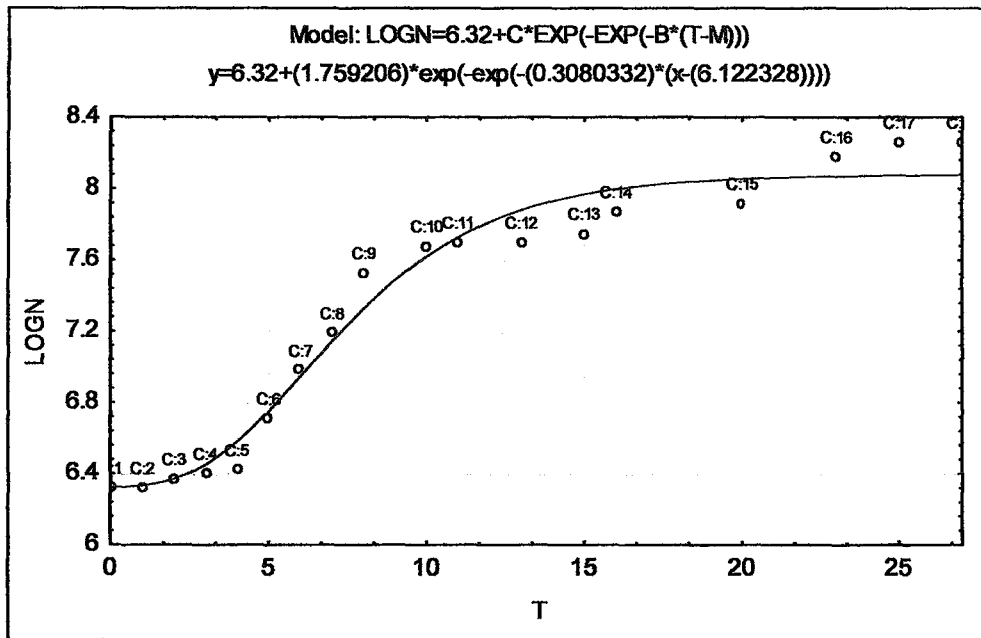


Figure B. 32. b. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and 200 mM sodium lactate.

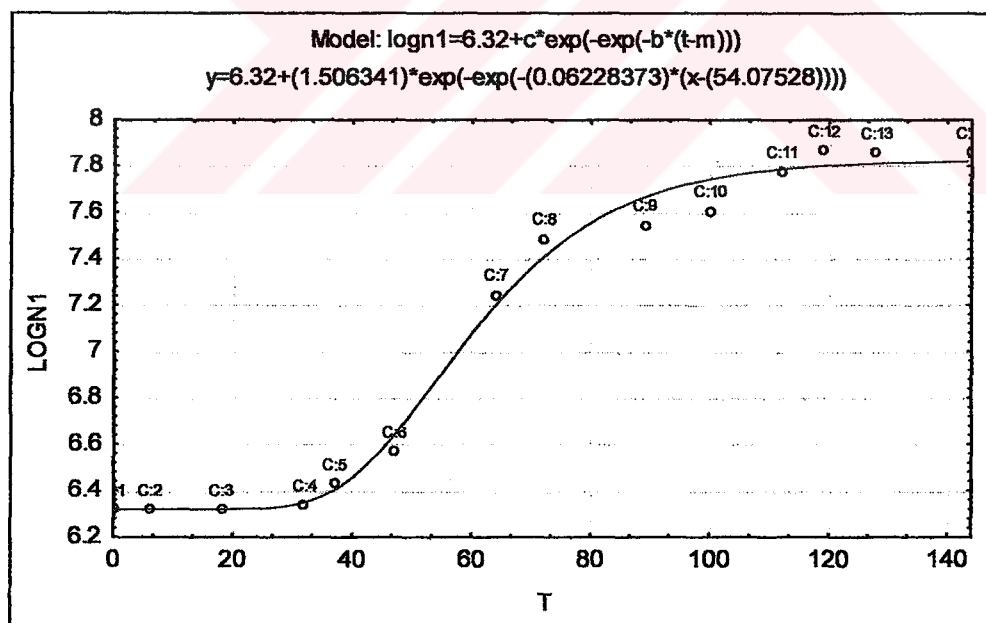


Figure B. 33. a. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and 400 mM sodium lactate.

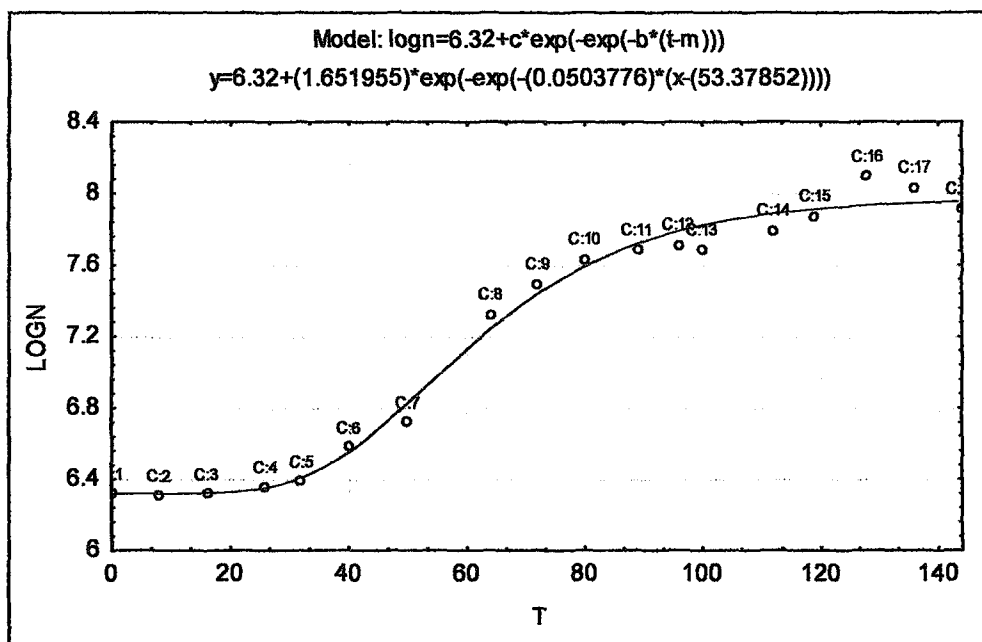


Figure B. 33. b. Growth curve of *B. cereus* at 26°C, 6.3 pH, 85 mM NaCl and 400 mM sodium lactate.

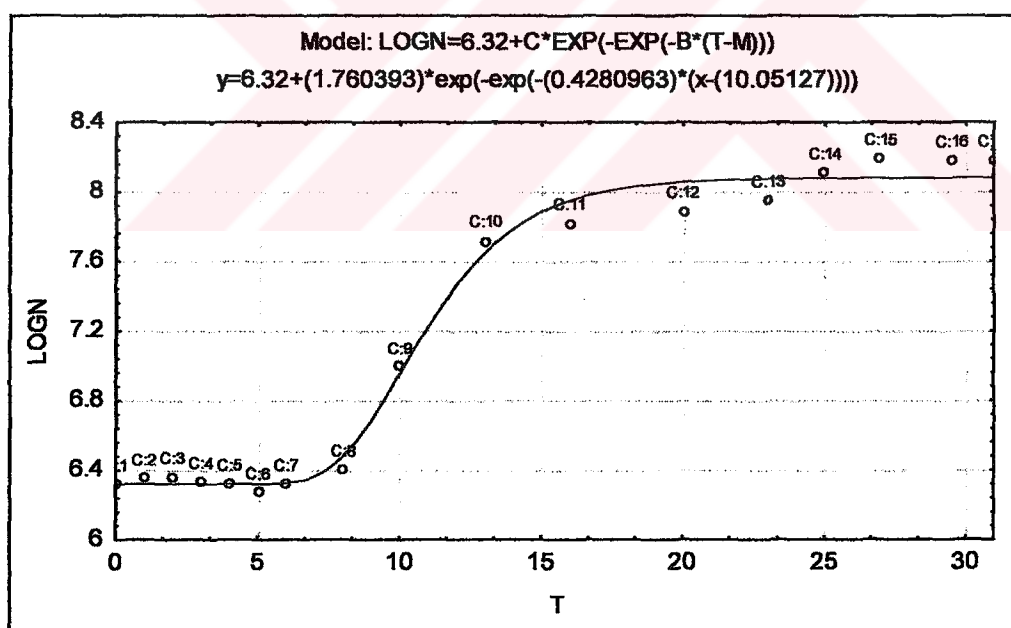


Figure B. 34. a. Growth curve of *B. cereus* at 26°C, 6.3 pH, 600 mM NaCl and without sodium lactate.

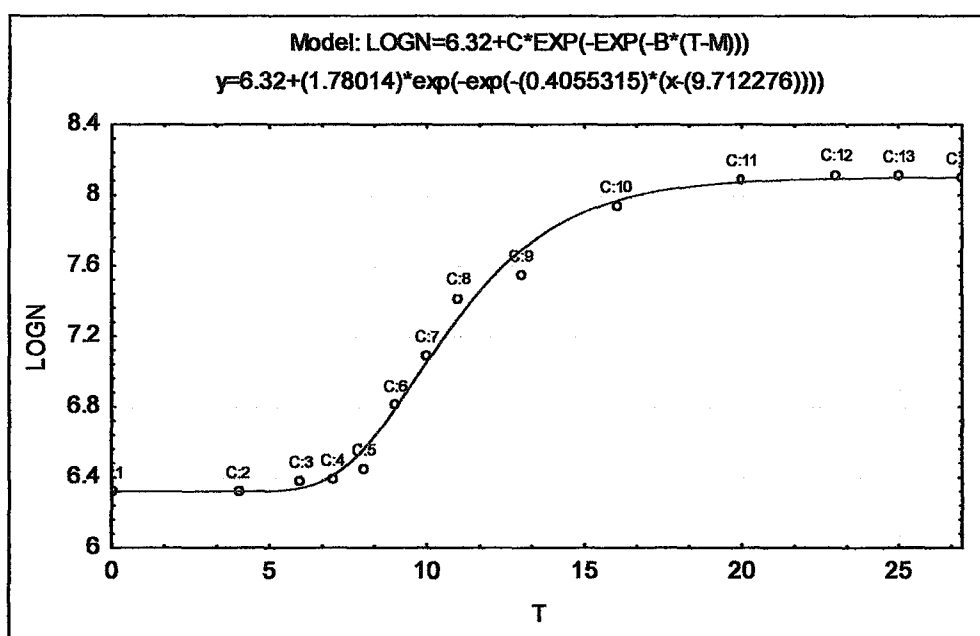


Figure B. 34. b. Growth curve of *B. cereus* at 26°C, 6.3 pH, 600 mM NaCl and without sodium lactate.

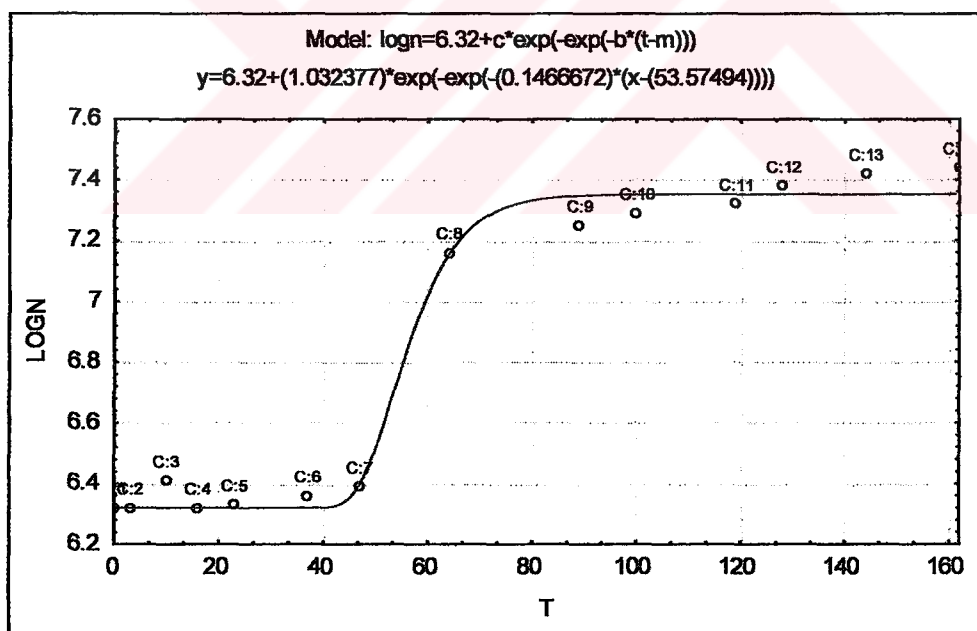


Figure B. 35. Growth curve of *B. cereus* at 26°C, 6.3 pH, 600 mM NaCl and 200 mM sodium lactate.

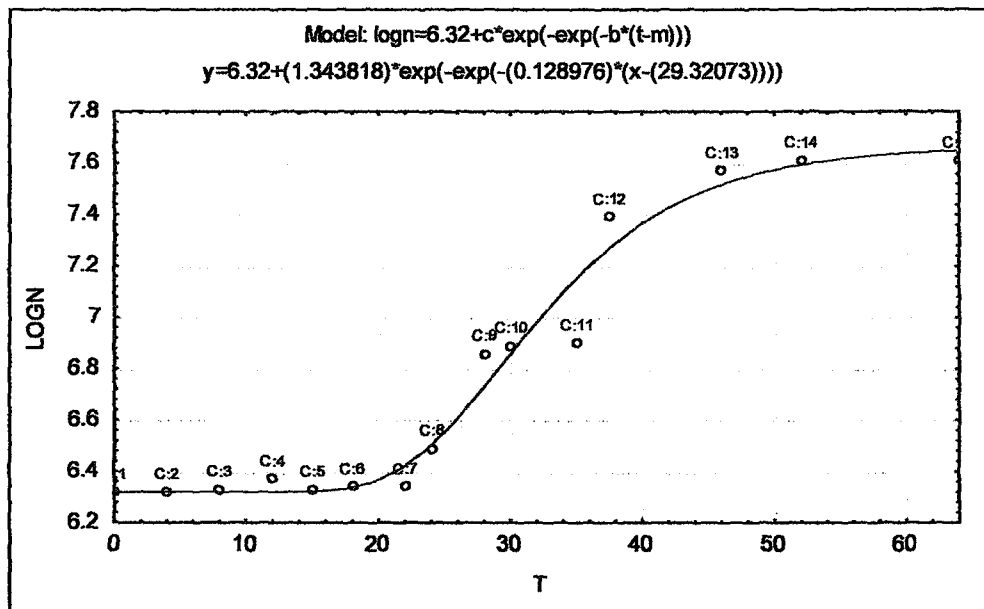


Figure B. 36. Growth curve of *B. cereus* at 26°C, 6.3 pH, 342 mM NaCl and 200 mM sodium lactate.

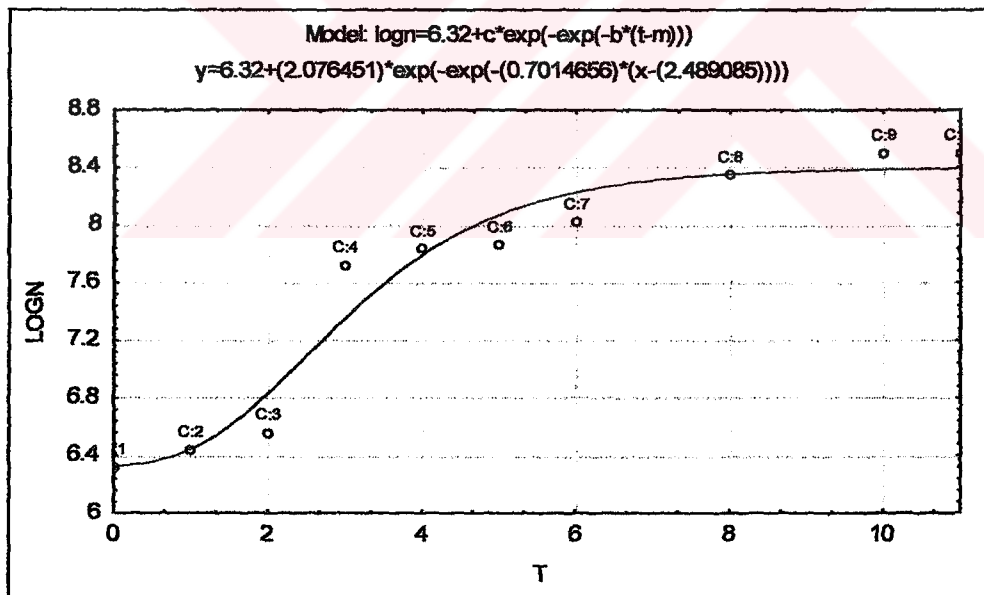


Figure B. 37. Growth curve of *B. cereus* at 26°C, 6.8 pH, 85 mM NaCl and without sodium lactate.

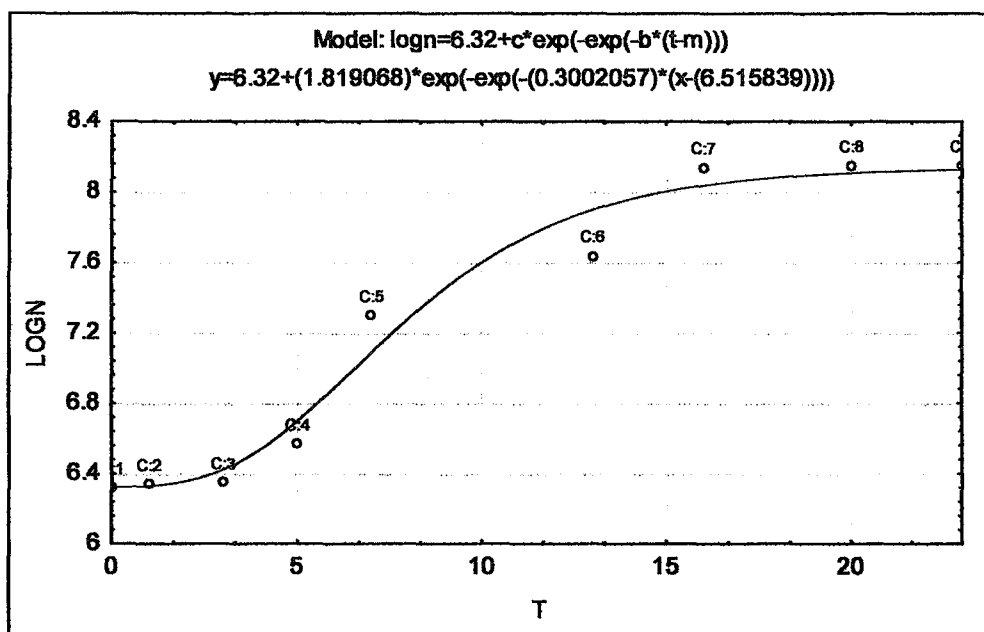


Figure B. 38. Growth curve of *B. cereus* at 26°C, 6.8 pH, 85 mM NaCl and 200 mM sodium lactate.

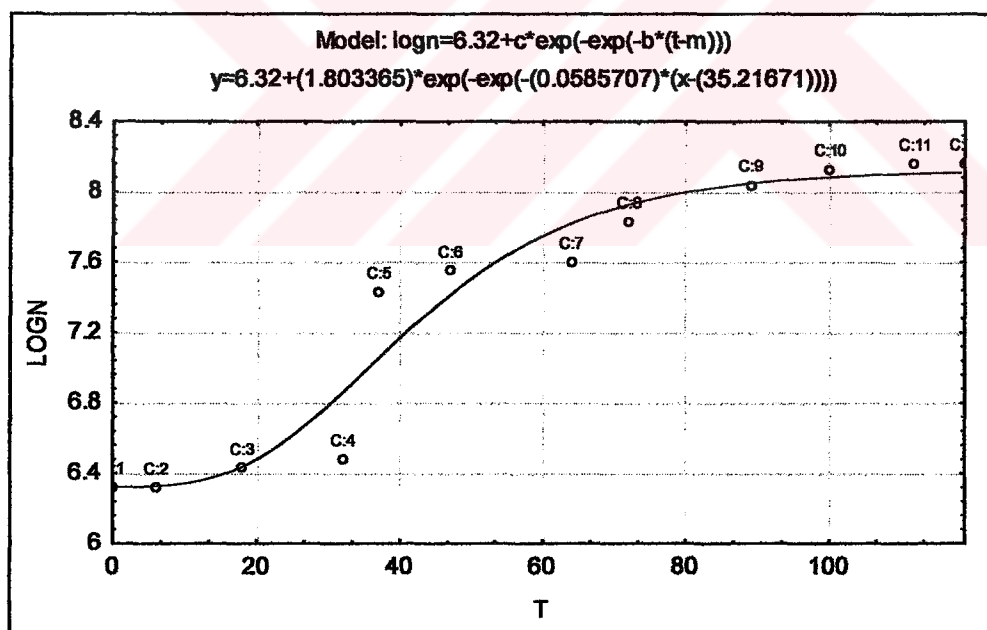


Figure B. 39. Growth curve of *B. cereus* at 26°C, 6.8 pH, 85 mM NaCl and 400 mM sodium lactate.

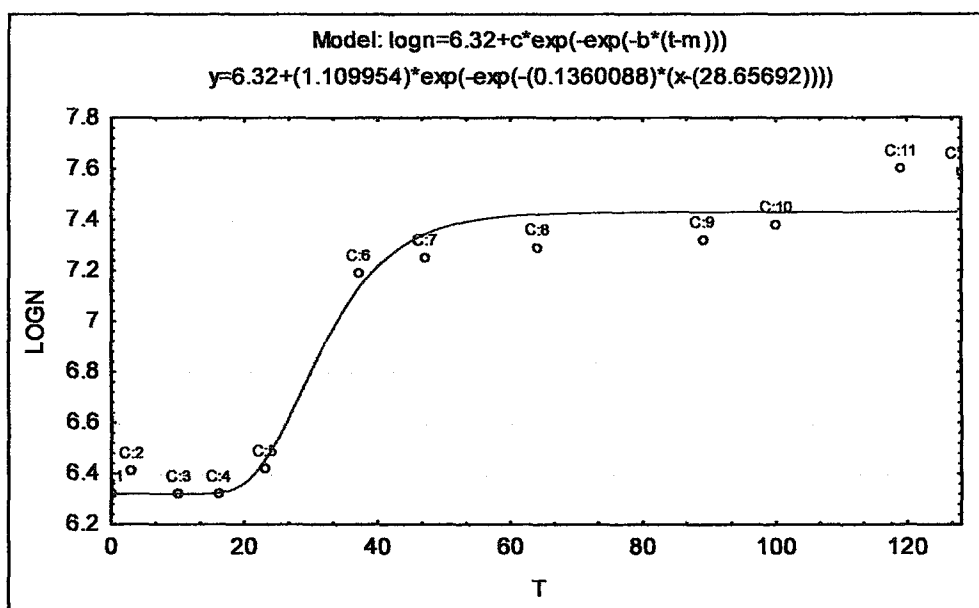


Figure B. 40. Growth curve of *B. cereus* at 26°C, 6.8 pH, 342 mM NaCl and 200 mM sodium lactate.

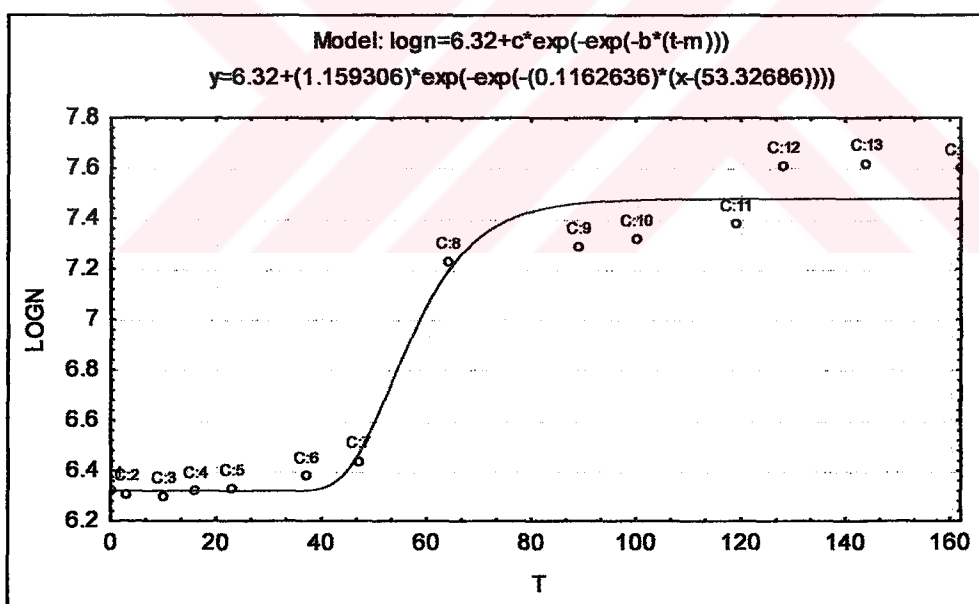


Figure B. 41. Growth curve of *B. cereus* at 26°C, 6.8 pH, 600 mM NaCl and 200 mM sodium lactate.

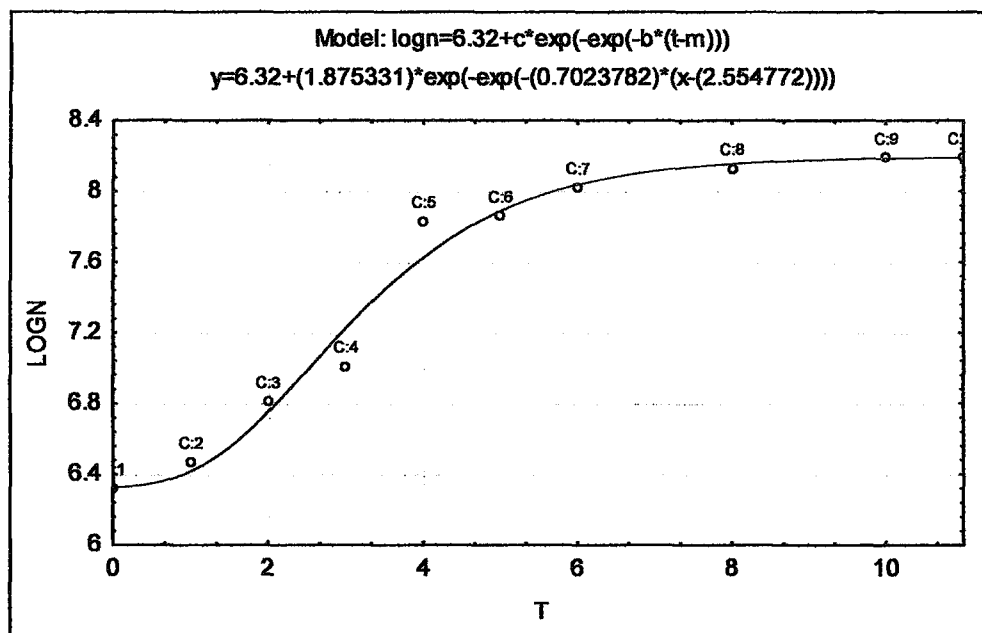


Figure B. 42. Growth curve of *B. cereus* at 26°C, 7.3 pH, 85 mM NaCl and without sodium lactate.

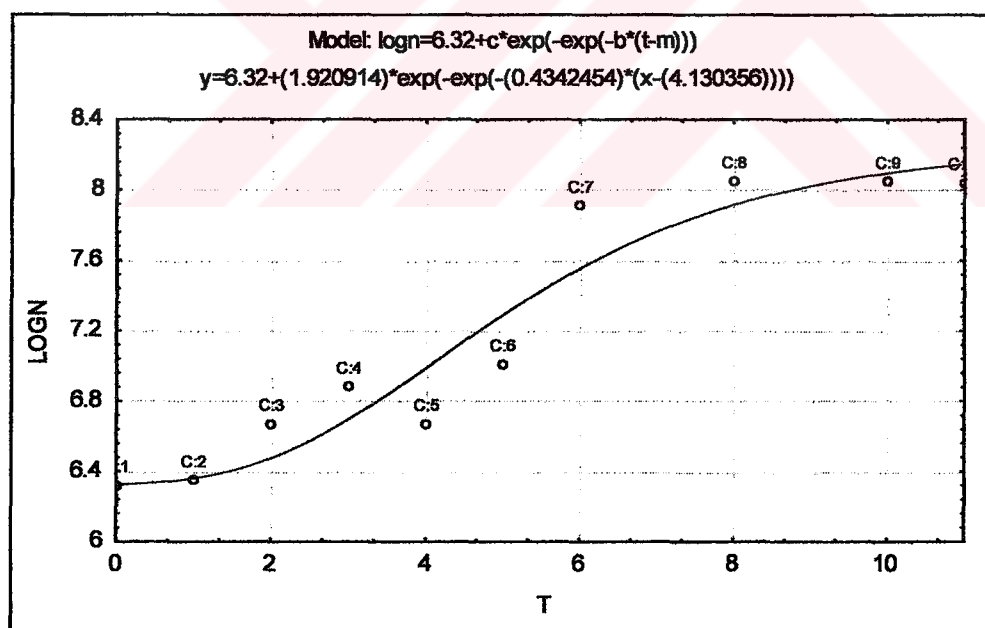


Figure B. 43. Growth curve of *B. cereus* at 26°C, 7.3 pH, 85 mM NaCl and 200 mM sodium lactate.

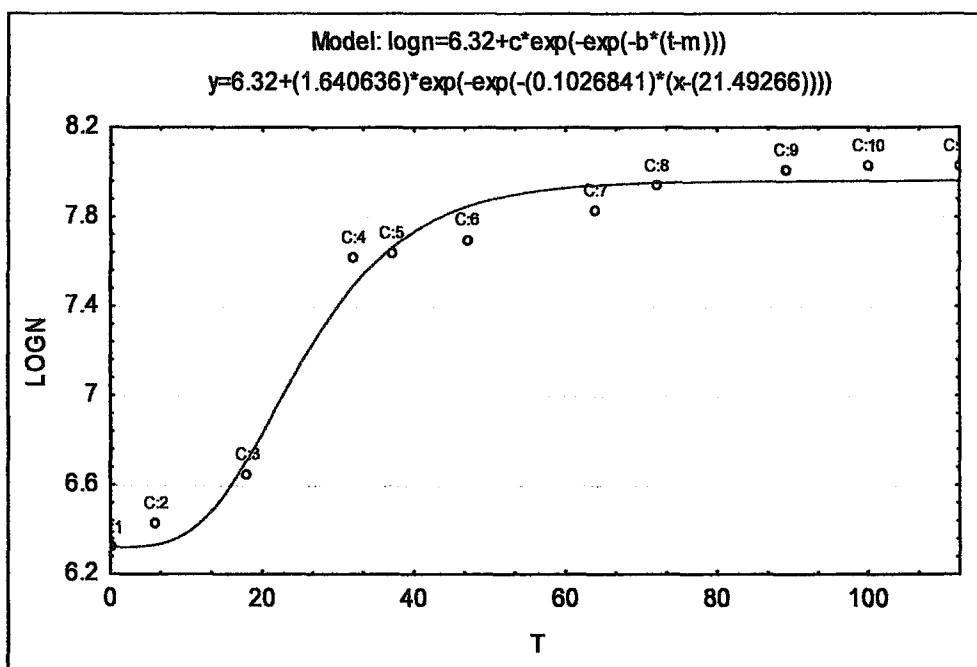


Figure B. 44. Growth curve of *B. cereus* at 26°C, 7.3 pH, 85 mM NaCl and 400 mM sodium lactate.

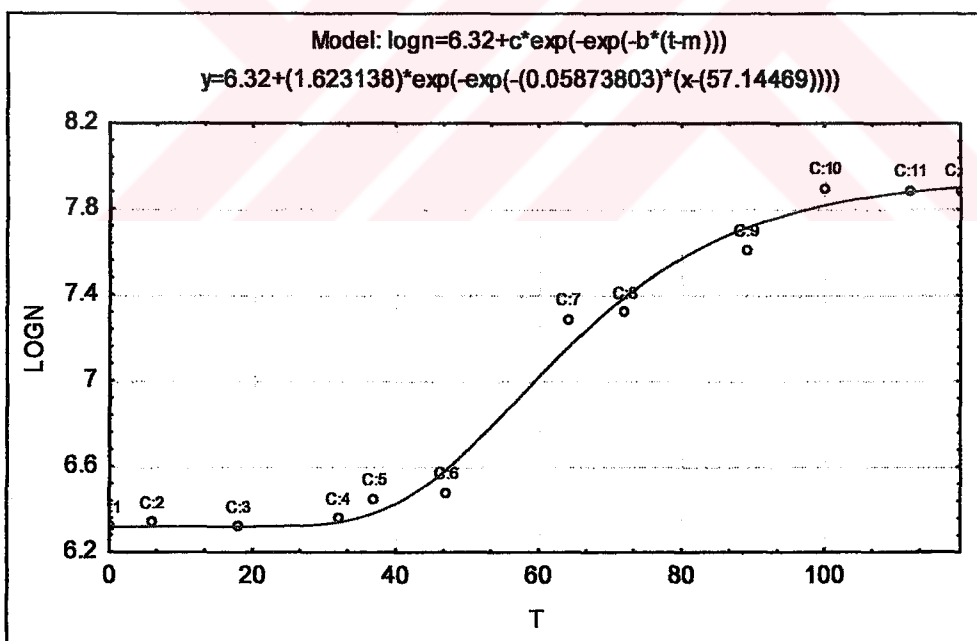


Figure B. 45. Growth curve of *B. cereus* at 26°C, 7.3 pH, 85 mM NaCl and 600 mM sodium lactate.

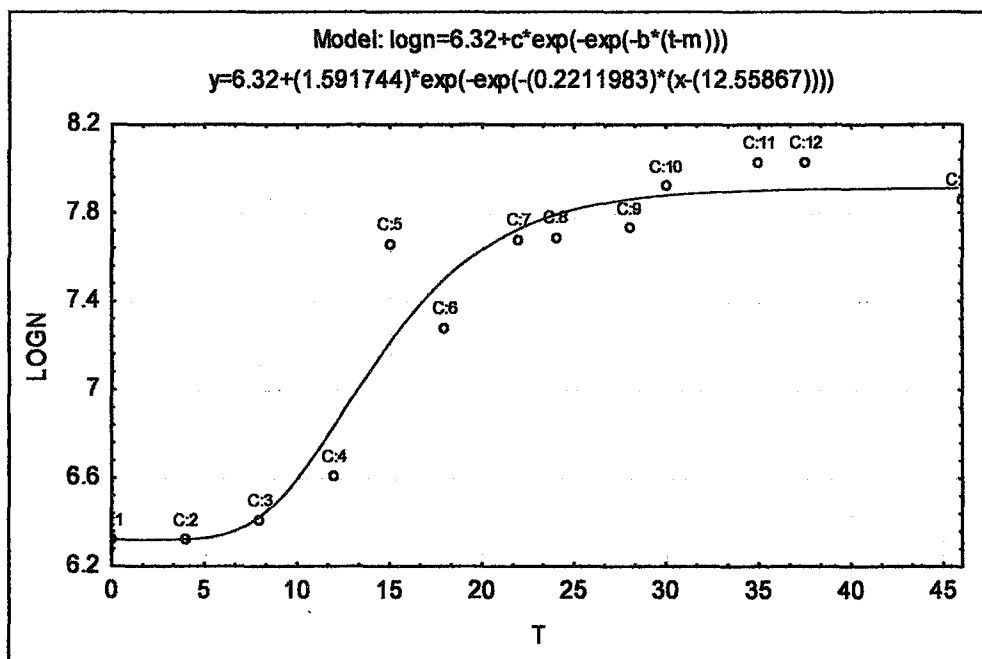


Figure B. 46. Growth curve of *B. cereus* at 26°C, 7.3 pH, 600 mM NaCl and without sodium lactate.

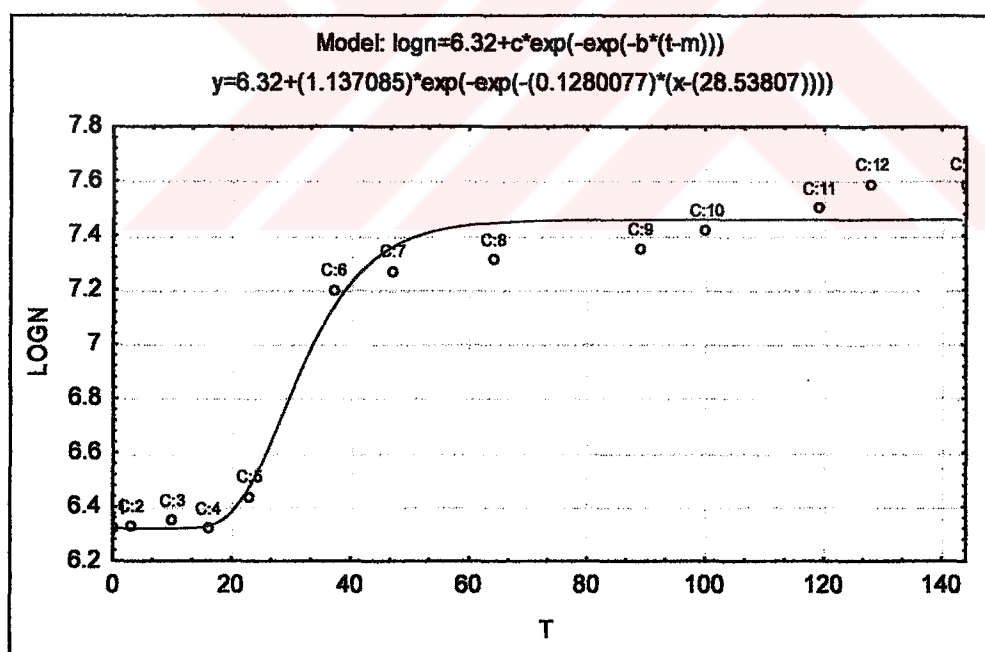


Figure B. 47. Growth curve of *B. cereus* at 26°C, 7.3 pH, 600 mM NaCl and 200 mM sodium lactate.

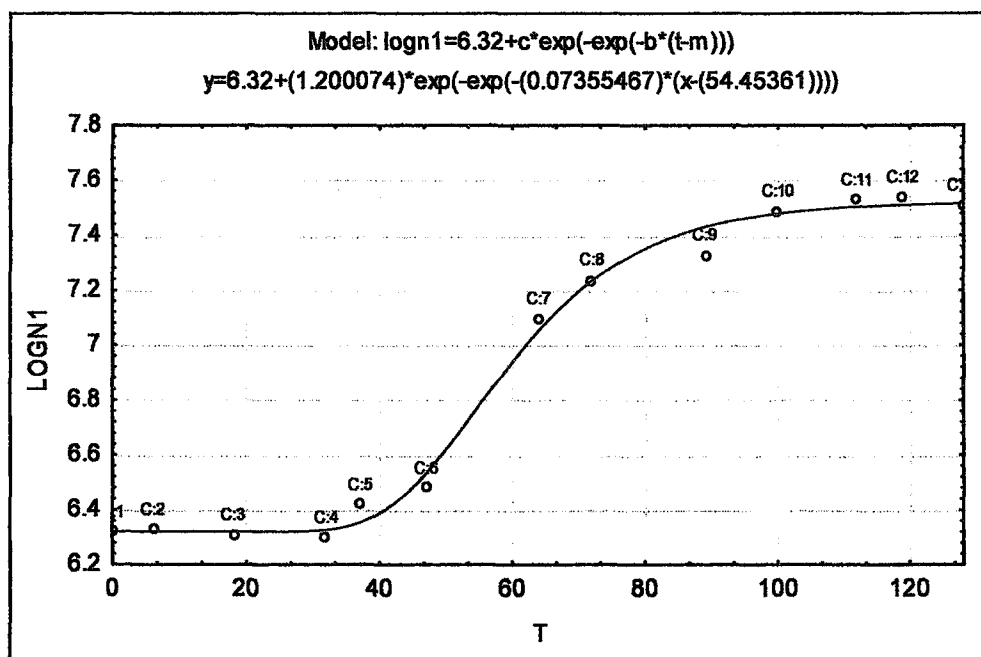


Figure B. 48. a. Growth curve of *B. cereus* at 26°C, 7.3 pH, 600 mM NaCl and 400 mM sodium lactate.

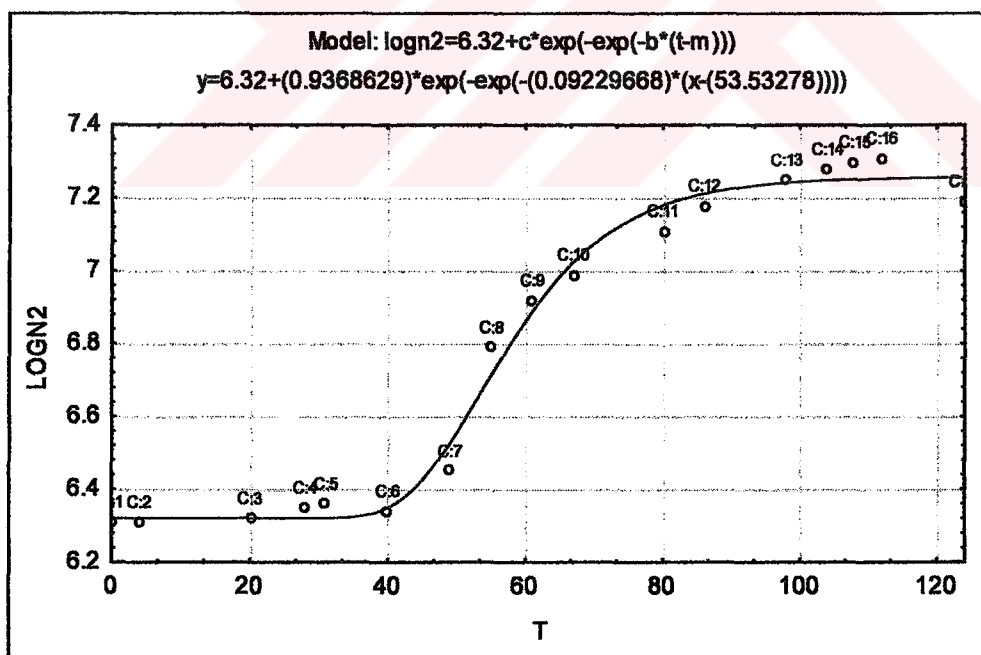


Figure B. 48. b. Growth curve of *B. cereus* at 26°C, 7.3 pH, 600 mM NaCl and 400 mM sodium lactate.

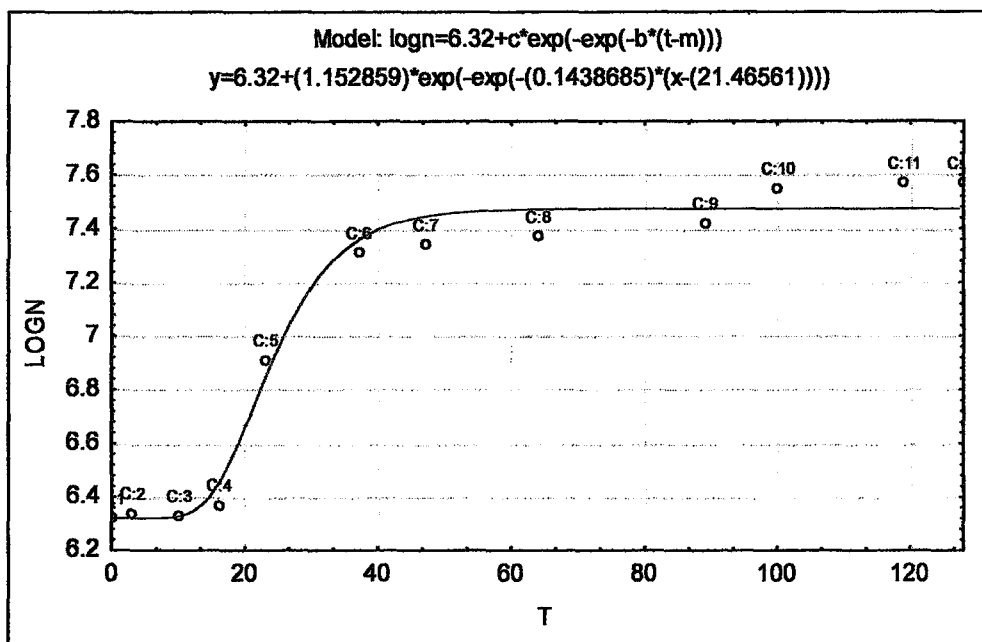


Figure B. 49. Growth curve of *B. cereus* at 26°C, 7.3 pH, 342 mM NaCl and 200 mM sodium lactate.

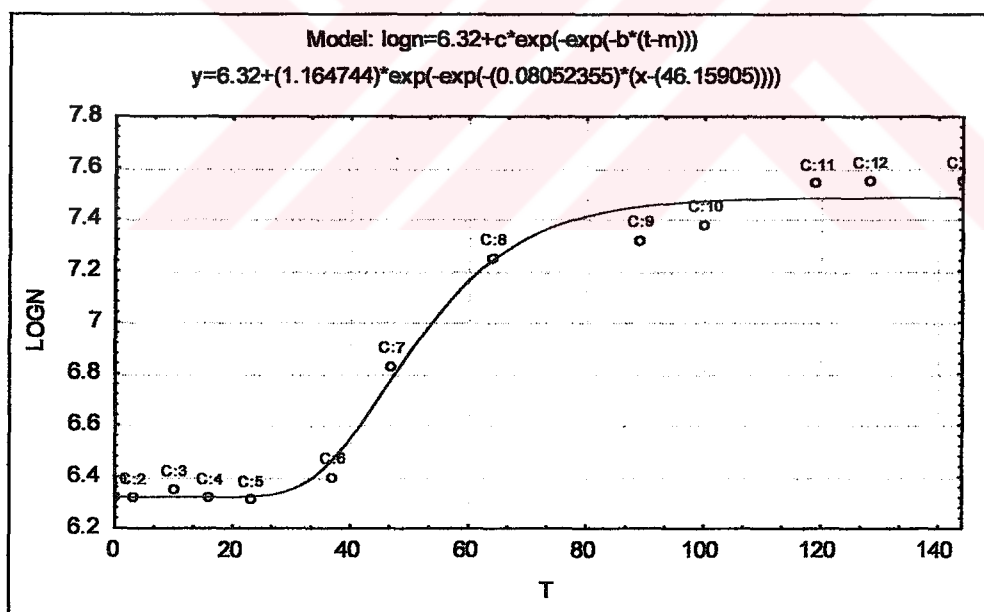


Figure B. 50. Growth curve of *B. cereus* at 26°C, 7.3 pH, 342 mM NaCl and 400 mM sodium lactate.

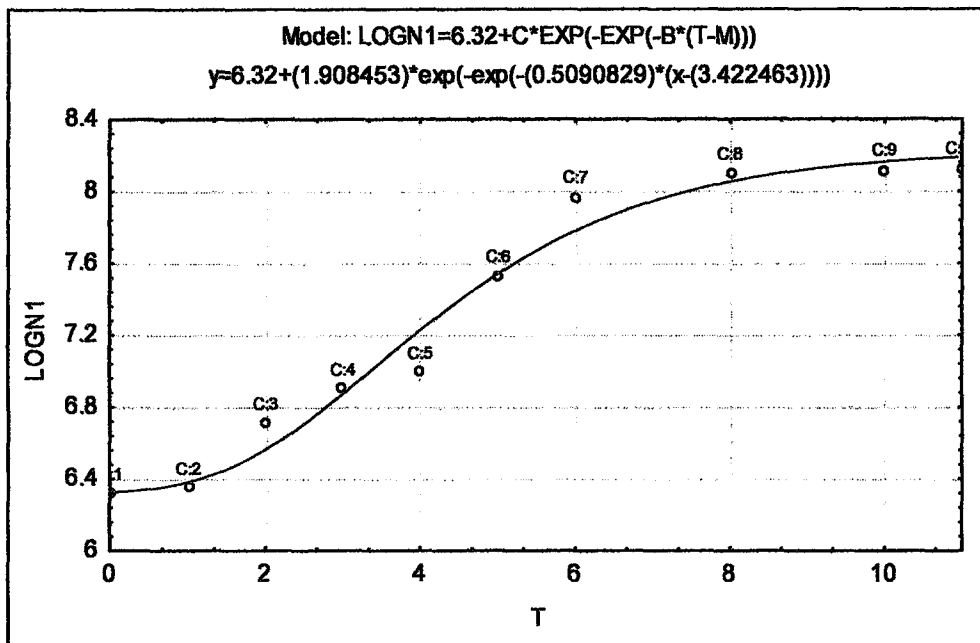


Figure B. 51. a. Growth curve of *B. cereus* at 32°C, 5.3 pH, 85 mM NaCl and without sodium lactate.

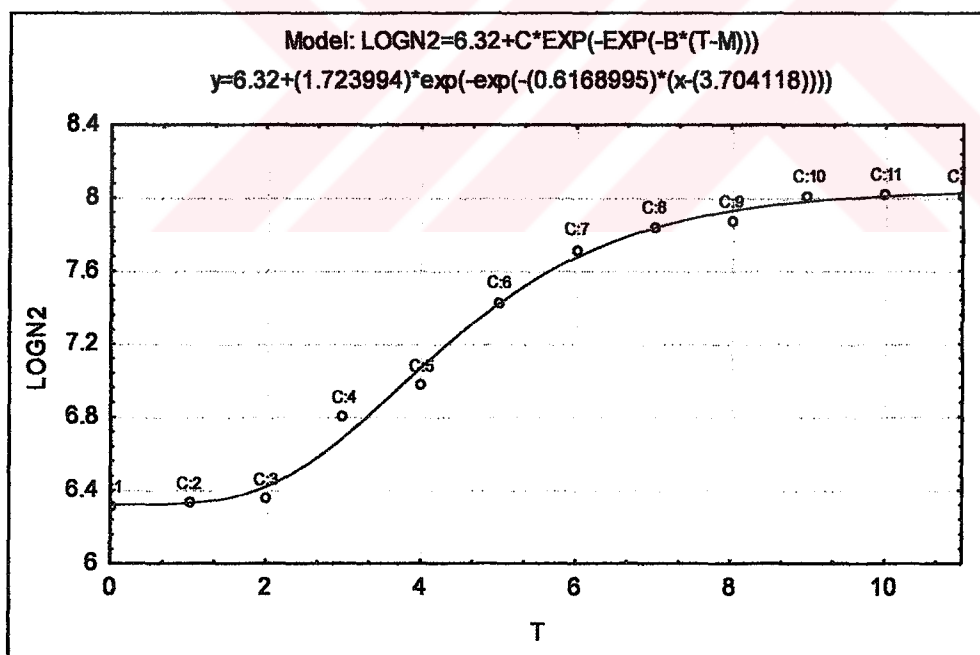


Figure B. 51. b. Growth curve of *B. cereus* at 32°C, 5.3 pH, 85 mM NaCl and without sodium lactate.

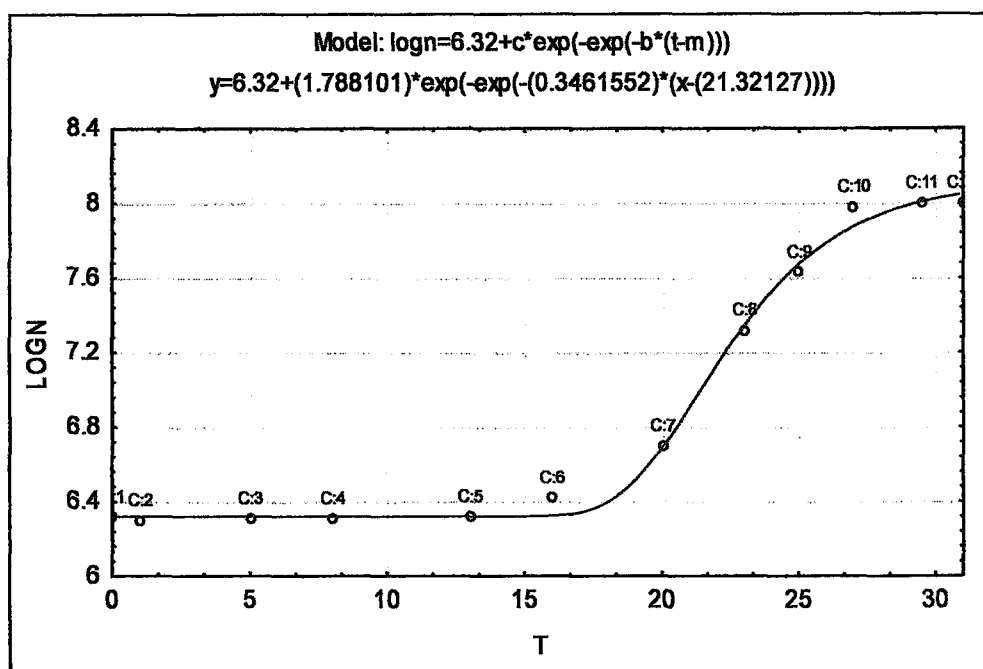


Figure B. 52. Growth curve of *B. cereus* at 32°C, 5.3 pH, 600 mM NaCl and without sodium lactate.

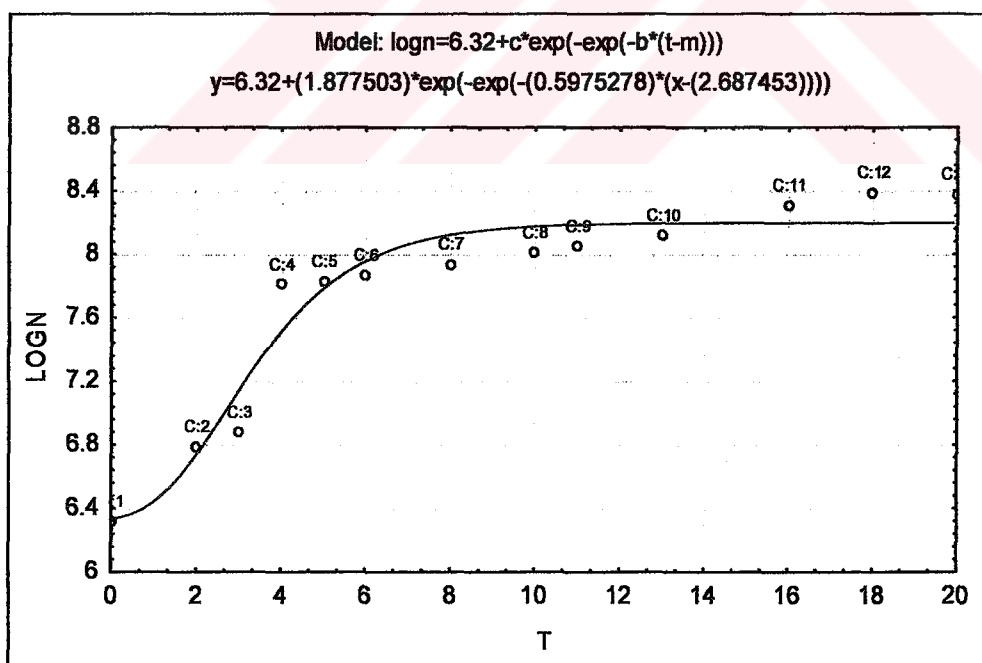


Figure B. 53. Growth curve of *B. cereus* at 32°C, 5.8 pH, 85 mM NaCl and without sodium lactate.

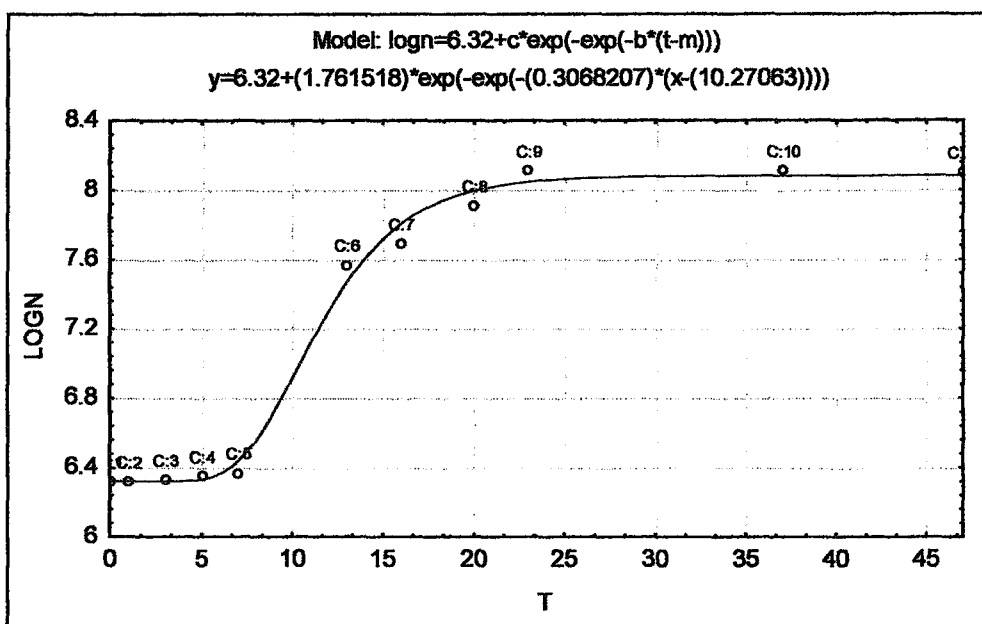


Figure B. 54. Growth curve of *B. cereus* at 32°C, 5.8 pH, 85 mM NaCl and 200 mM sodium lactate.

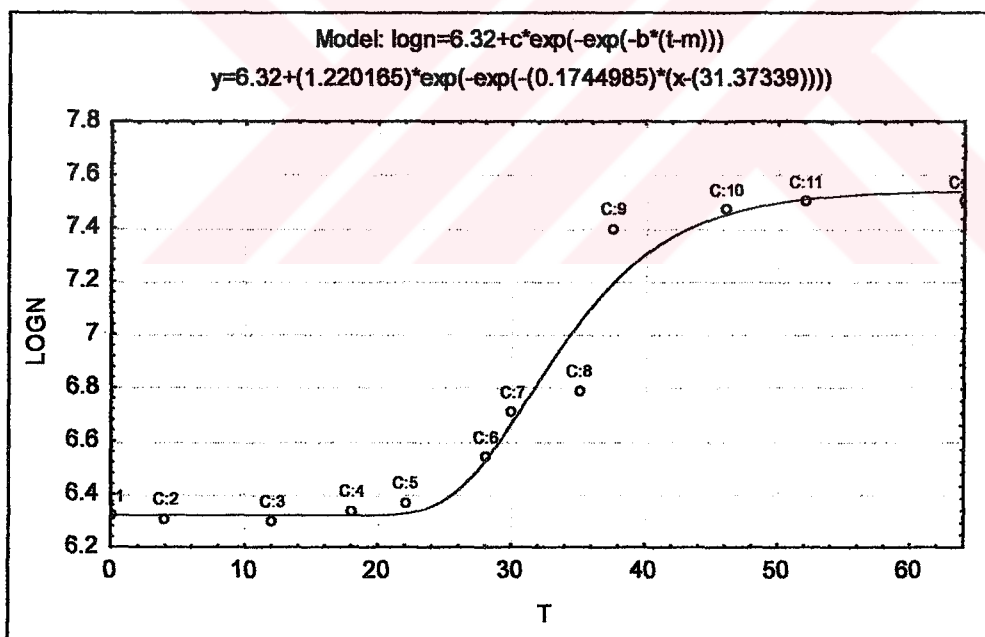


Figure B. 55. Growth curve of *B. cereus* at 32°C, 5.8 pH, 342 mM NaCl and 200 mM sodium lactate.

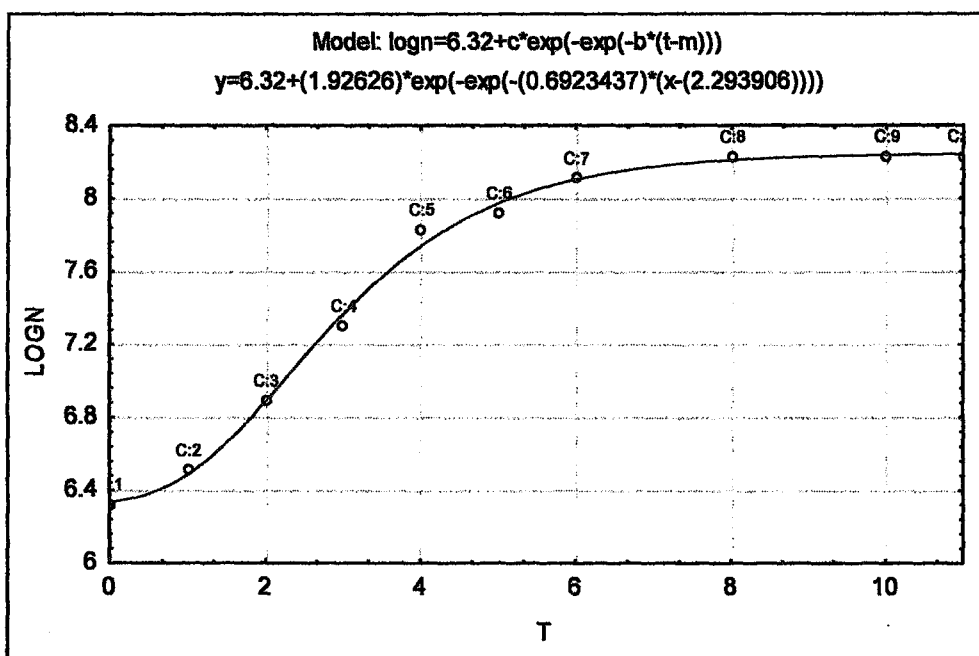


Figure B. 56. Growth curve of *B. cereus* at 32°C, 6.3 pH, 85 mM NaCl and without sodium lactate.

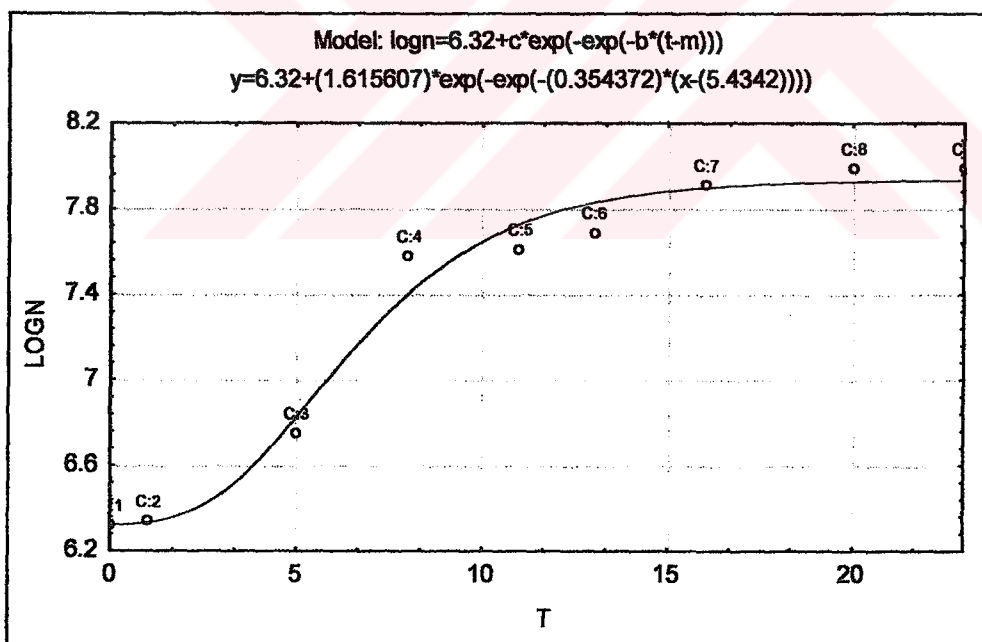


Figure B. 57. Growth curve of *B. cereus* at 32°C, 6.3 pH, 85 mM NaCl and 200 mM sodium lactate.

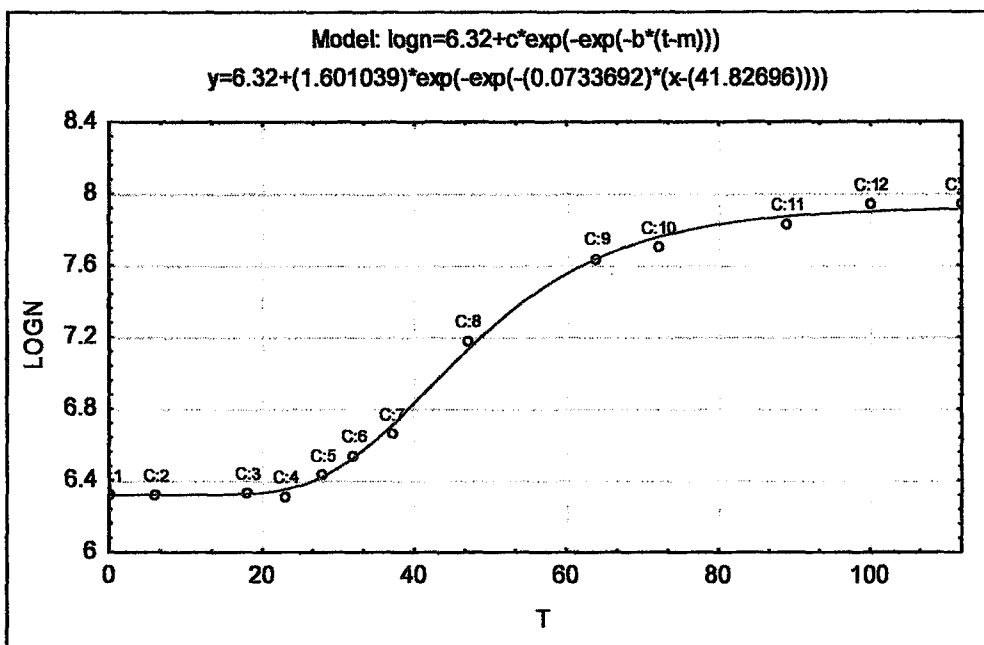


Figure B. 58. Growth curve of *B. cereus* at 32°C, 6.3 pH, 85 mM NaCl and 400 mM sodium lactate.

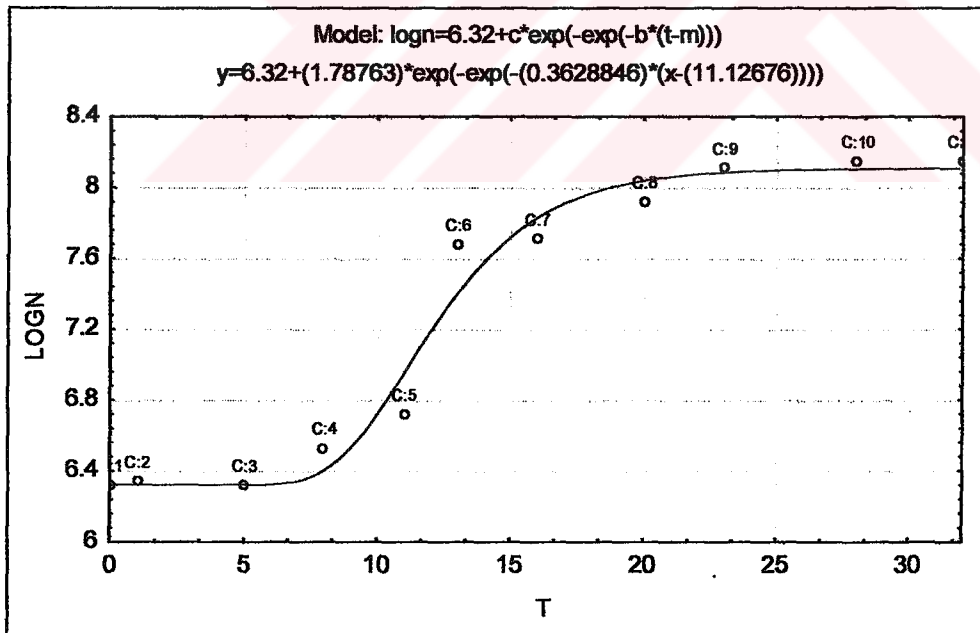


Figure B. 59. Growth curve of *B. cereus* at 32°C, 6.3 pH, 600 mM NaCl and without sodium lactate.

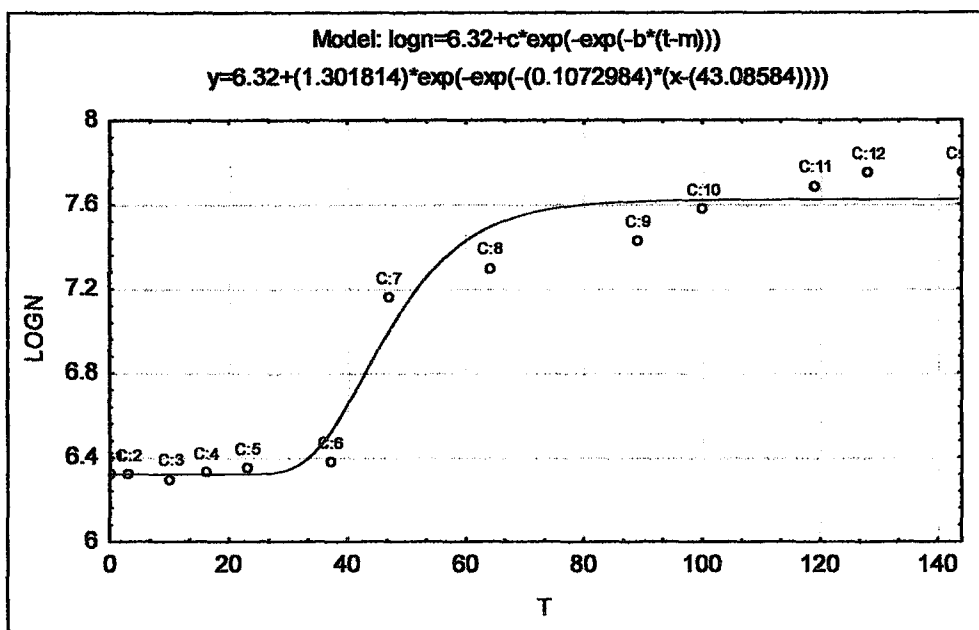


Figure B. 60. Growth curve of *B. cereus* at 32°C, 6.3 pH, 600 mM NaCl and 200 mM sodium lactate.

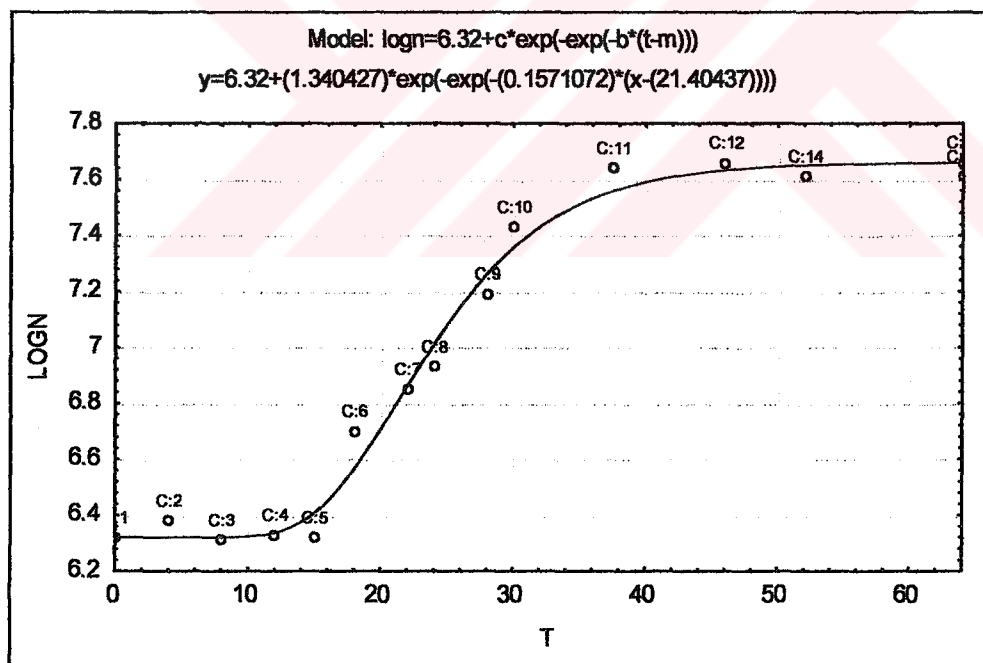


Figure B. 61. Growth curve of *B. cereus* at 32°C, 6.3 pH, 342 mM NaCl and 200 mM sodium lactate.

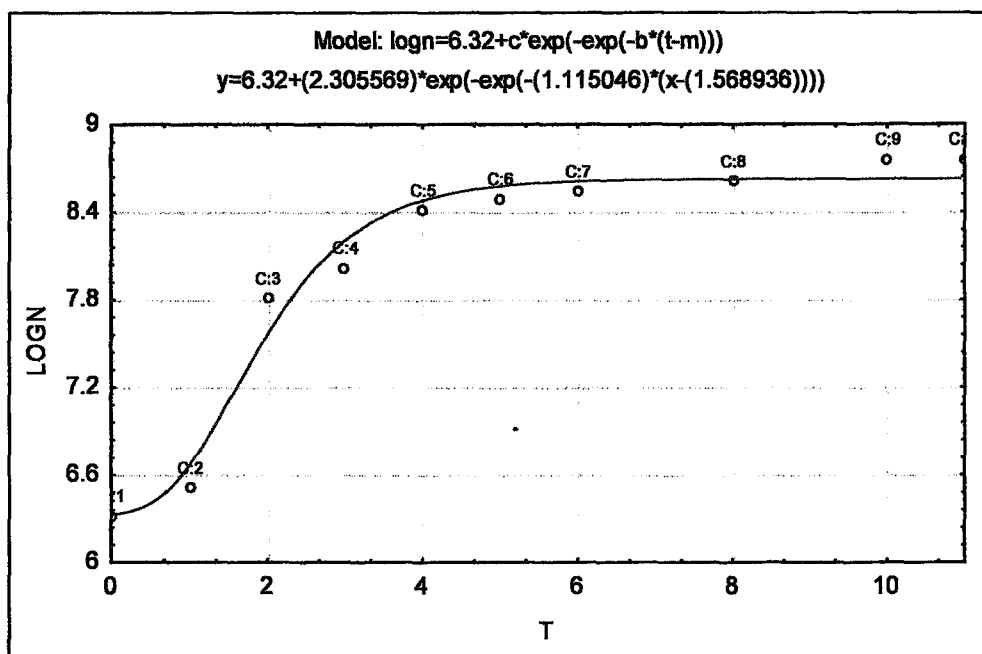


Figure B. 62. Growth curve of *B. cereus* at 32°C, 6.8 pH, 85 mM NaCl and without sodium lactate.

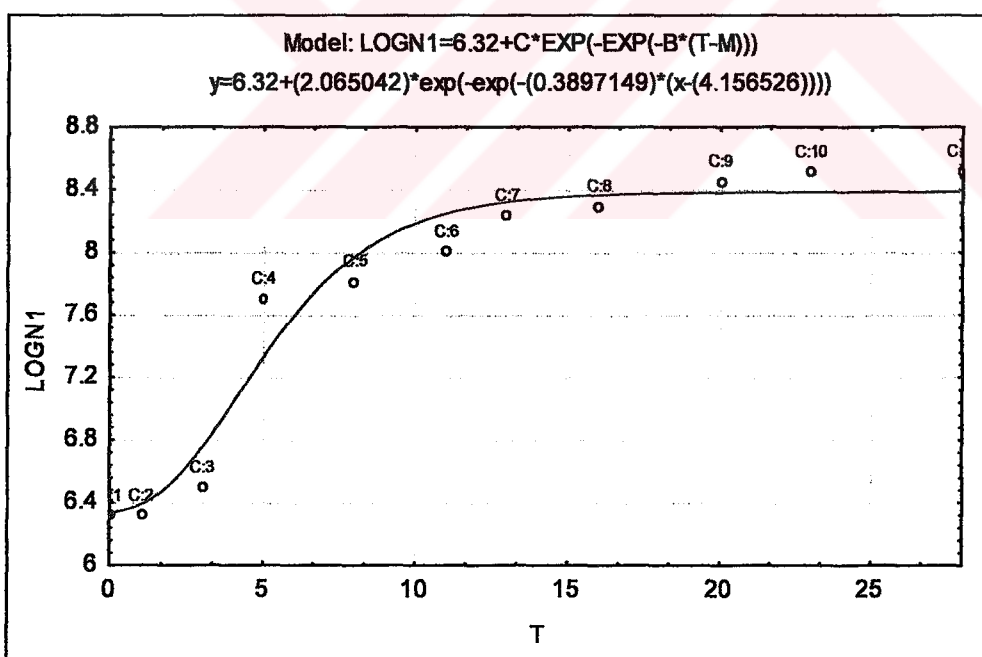


Figure B. 63. a. Growth curve of *B. cereus* at 32°C, 6.8 pH, 85 mM NaCl and 200 mM sodium lactate.

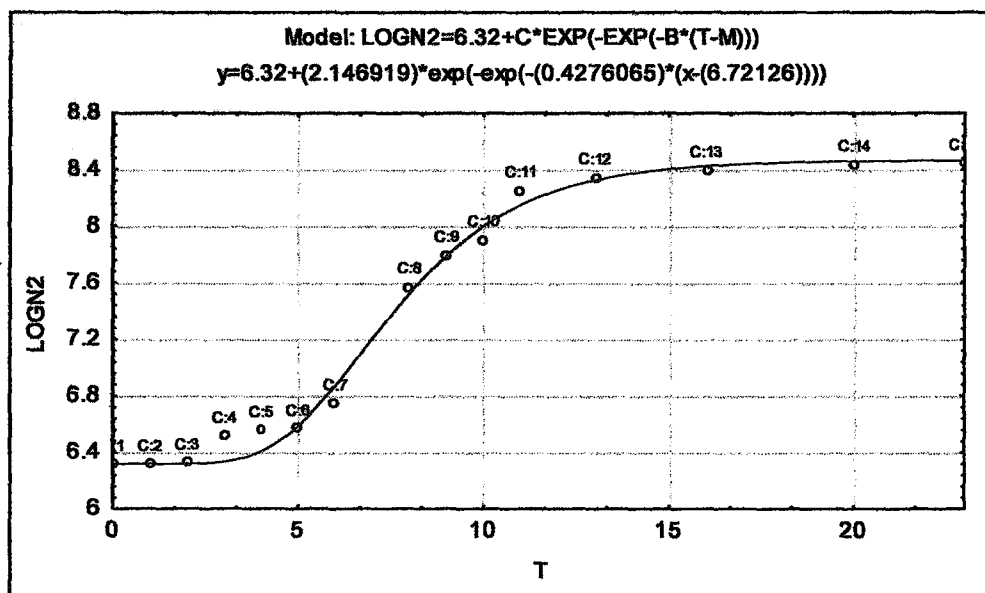


Figure B. 63. b. Growth curve of *B. cereus* at 32°C, 6.8 pH, 85 mM NaCl and 200 mM sodium lactate.

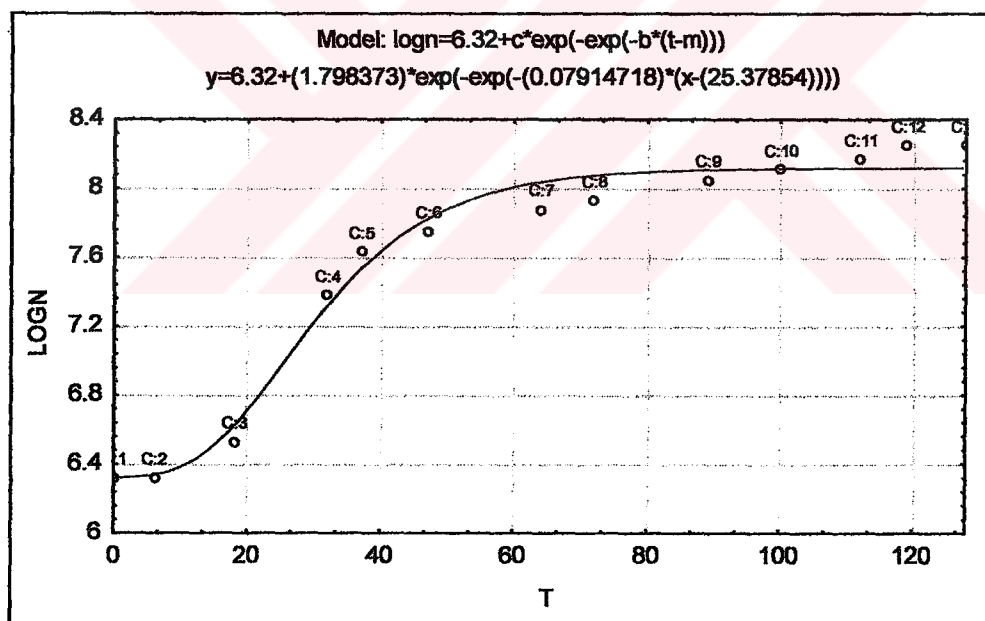


Figure B. 64. Growth curve of *B. cereus* at 32°C, 6.8 pH, 85 mM NaCl and 400 mM sodium lactate.

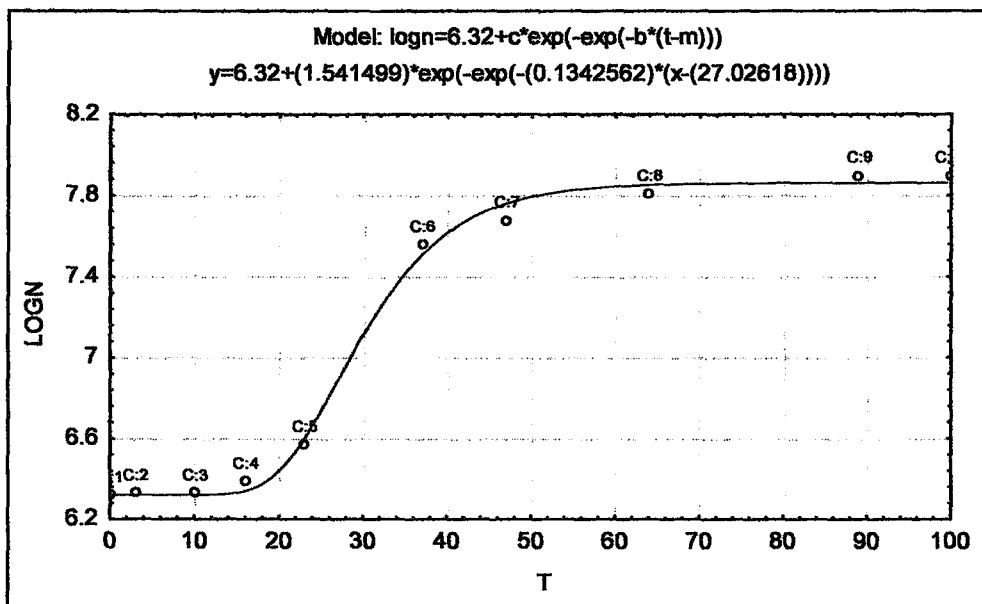


Figure B. 65. Growth curve of *B. cereus* at 32°C, 6.8 pH, 342 mM NaCl and 200 mM sodium lactate.

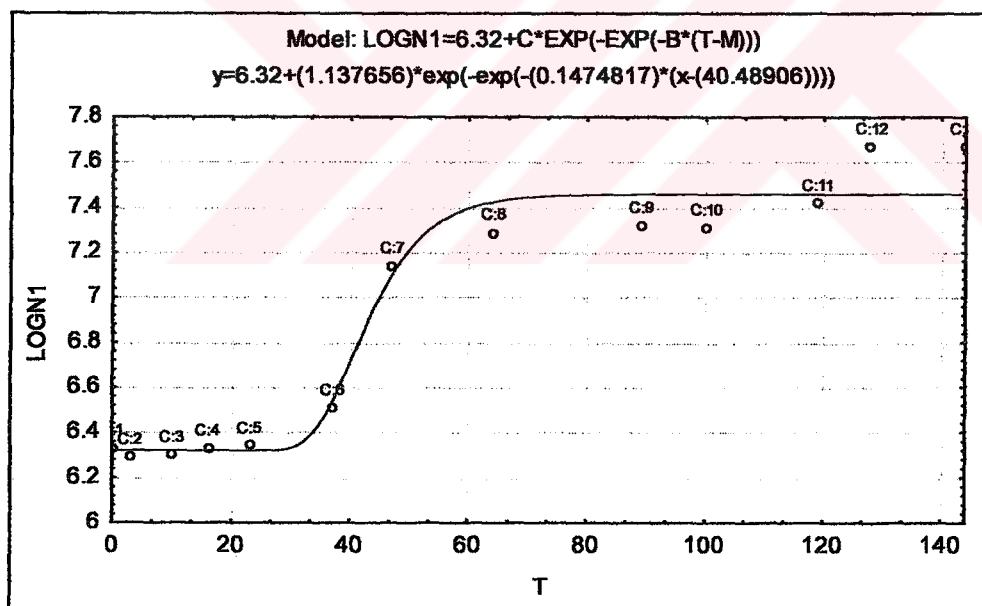


Figure B. 66. a. Growth curve of *B. cereus* at 32°C, 6.8 pH, 600 mM NaCl and 200 mM sodium lactate.

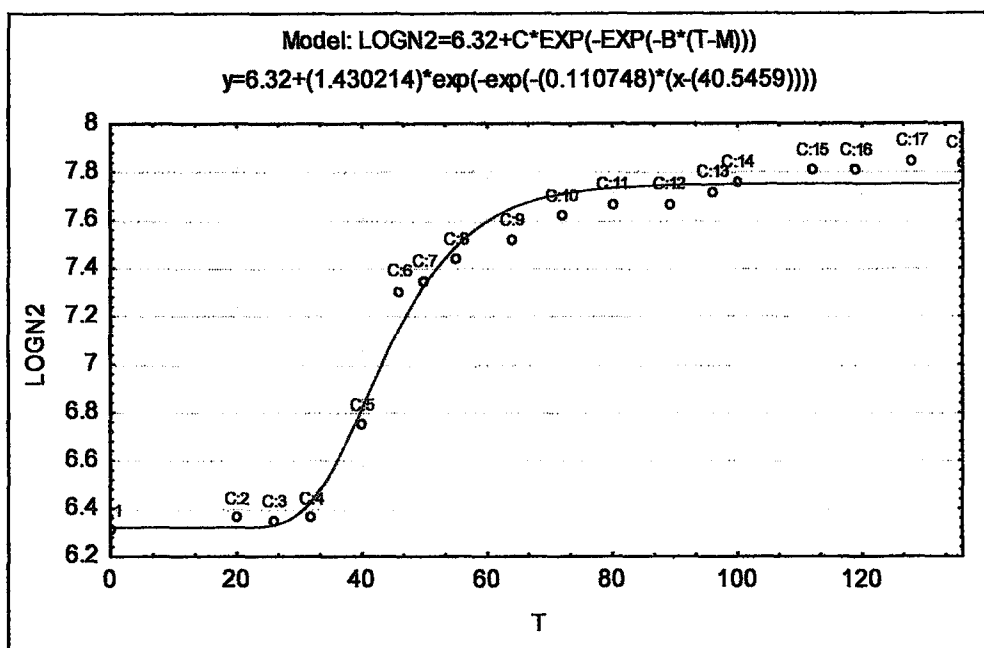


Figure B. 66. b. Growth curve of *B. cereus* at 32°C, 6.8 pH, 600 mM NaCl and 200 mM sodium lactate.

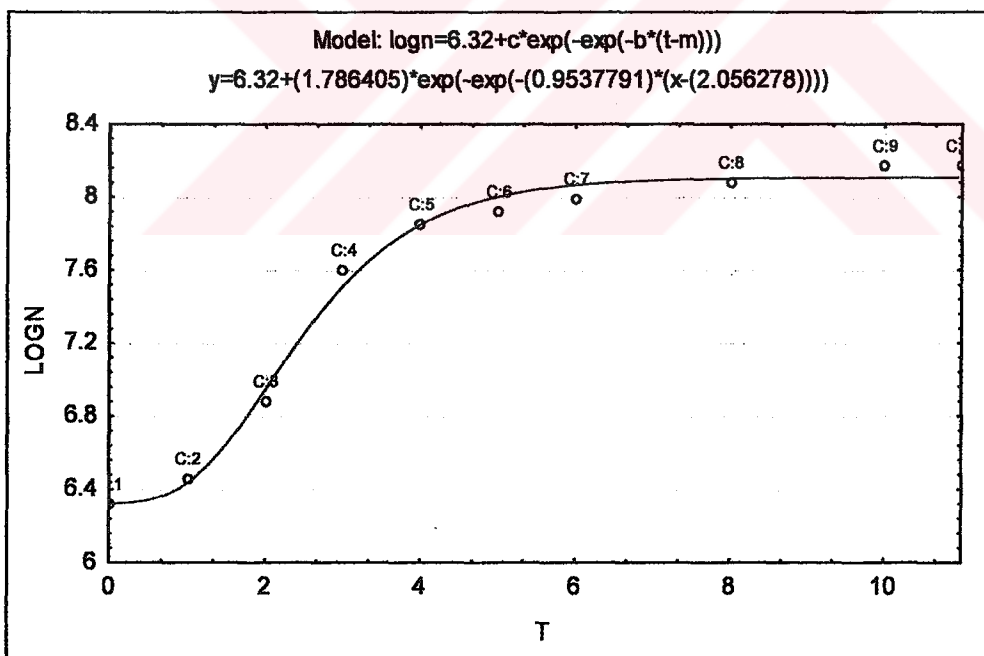


Figure B. 67. Growth curve of *B. cereus* at 32°C, 7.3 pH, 85 mM NaCl and without sodium lactate.

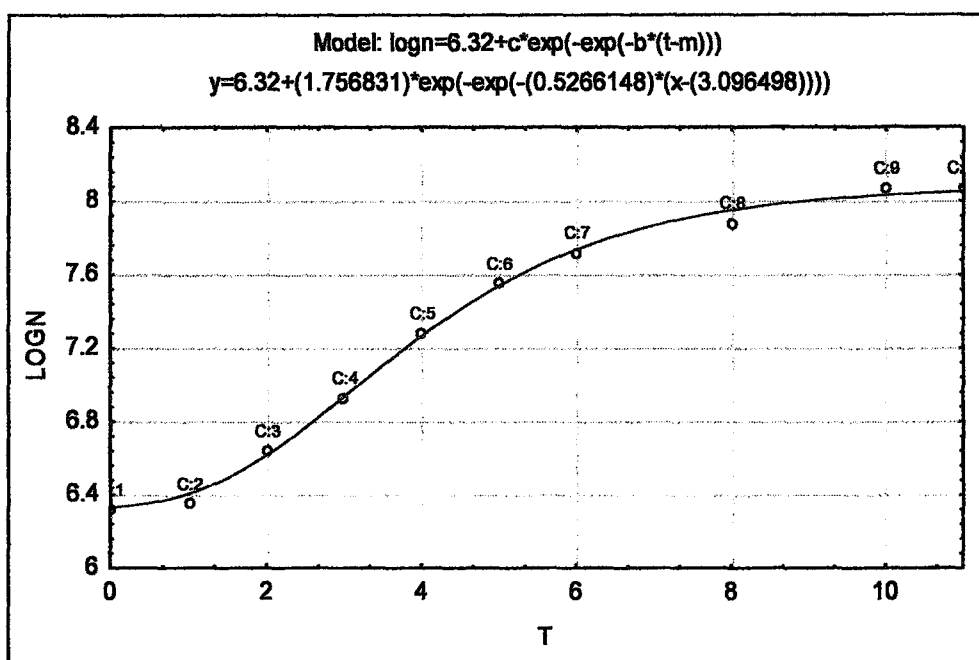


Figure B. 68. Growth curve of *B. cereus* at 32°C, 7.3 pH, 85 mM NaCl and 200 mM sodium lactate.

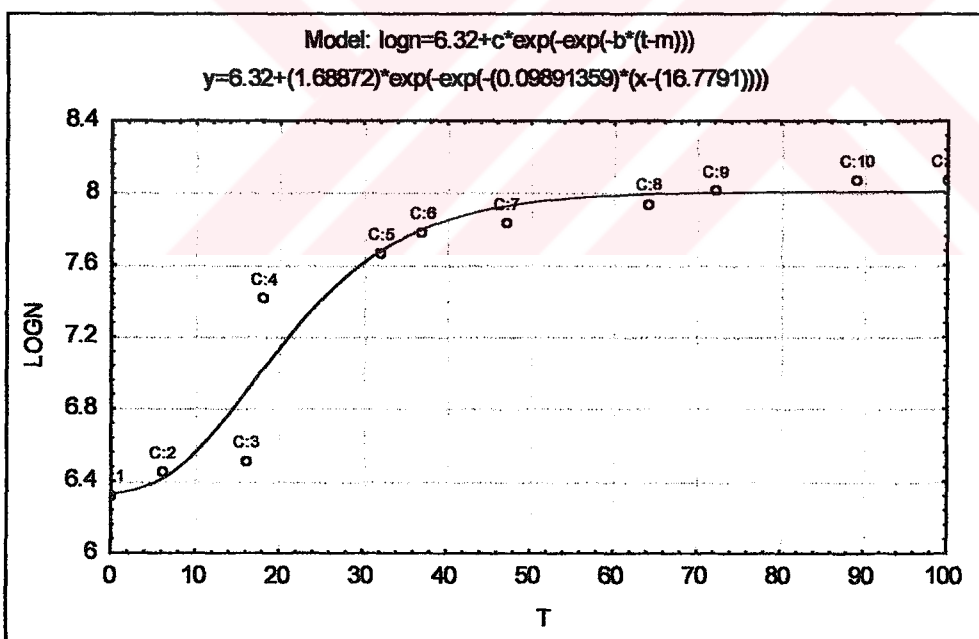


Figure B. 69. Growth curve of *B. cereus* at 32°C, 7.3 pH, 85 mM NaCl and 400 mM sodium lactate.

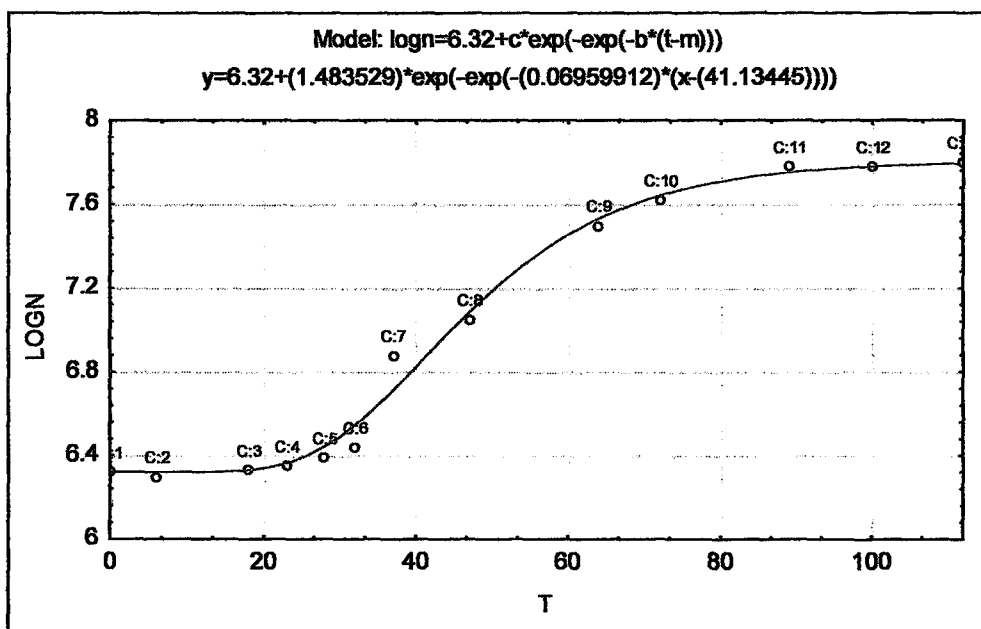


Figure B. 70. Growth curve of *B. cereus* at 32°C, 7.3 pH, 85 mM NaCl and 600 mM sodium lactate.

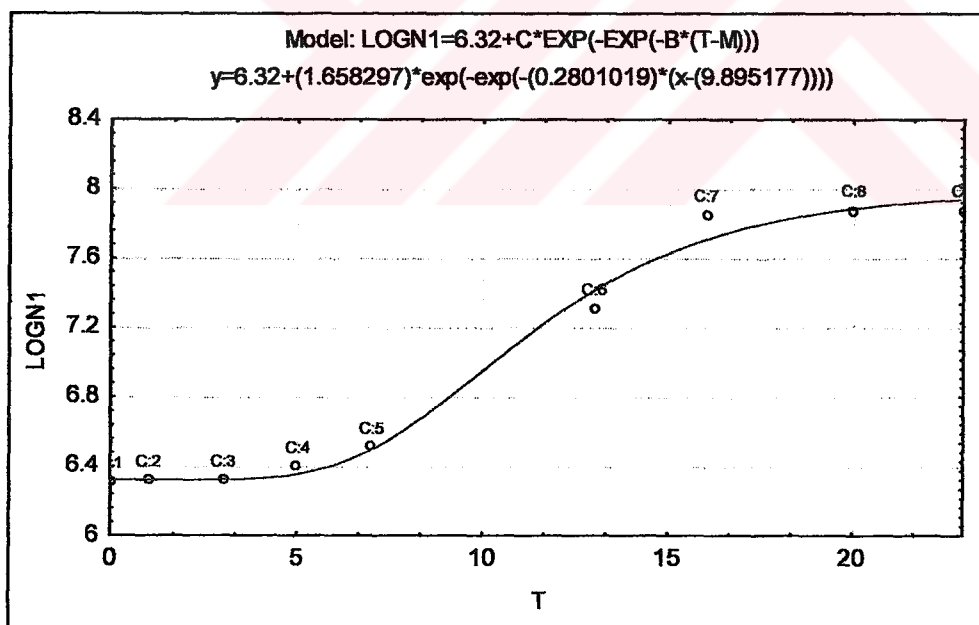


Figure B. 71. a. Growth curve of *B. cereus* at 32°C, 7.3 pH, 600 mM NaCl and without sodium lactate.

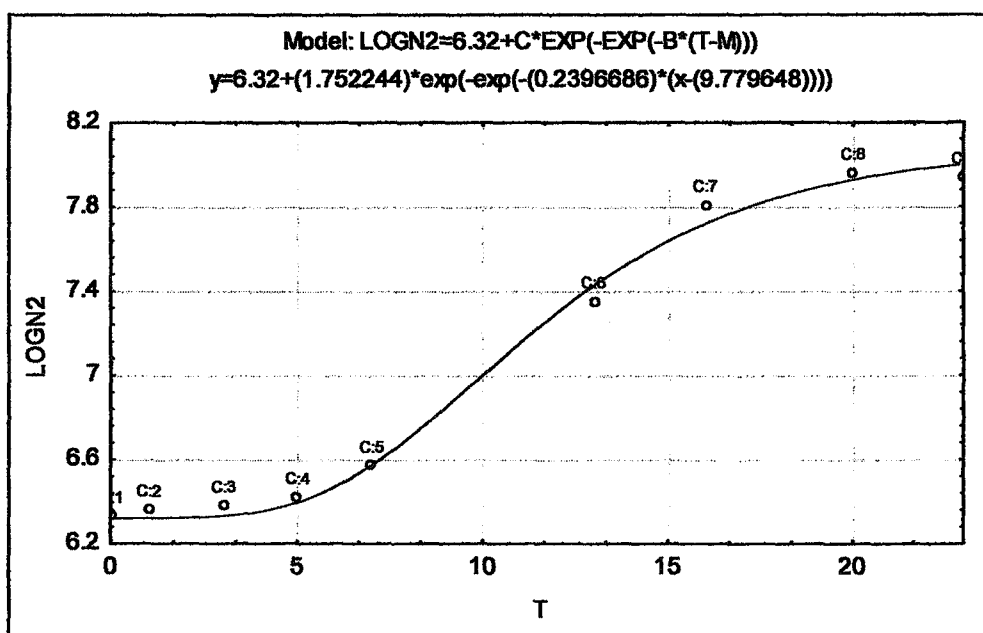


Figure B. 71. b. Growth curve of *B. cereus* at 32°C, 7.3 pH, 600 mM NaCl and without sodium lactate.

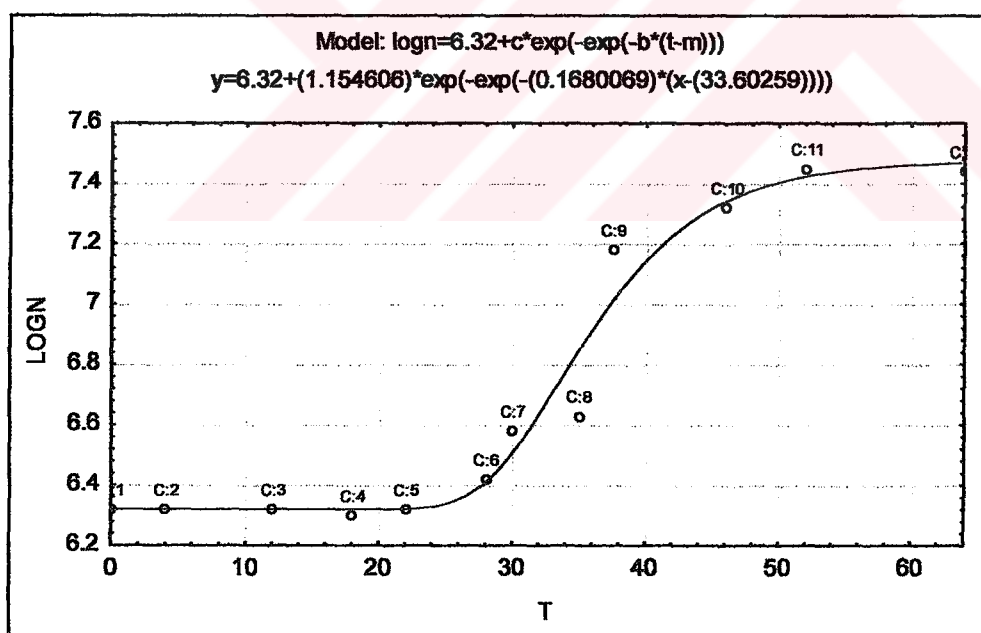


Figure B. 72. Growth curve of *B. cereus* at 32°C, 7.3 pH, 600 mM NaCl and 200 mM sodium lactate.

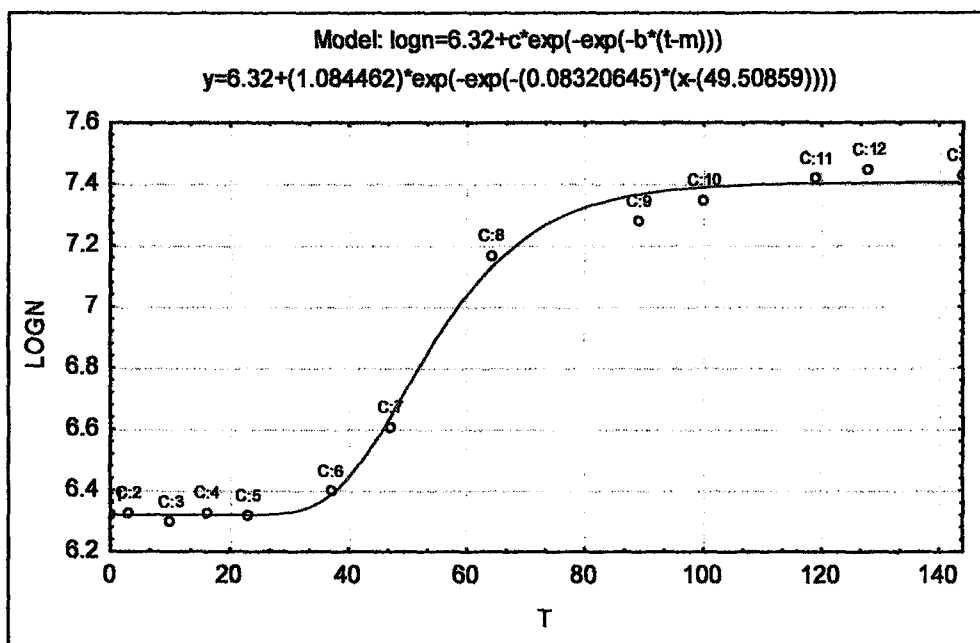


Figure B. 73. Growth curve of *B. cereus* at 32°C, 7.3 pH, 600 mM NaCl and 400 mM sodium lactate.

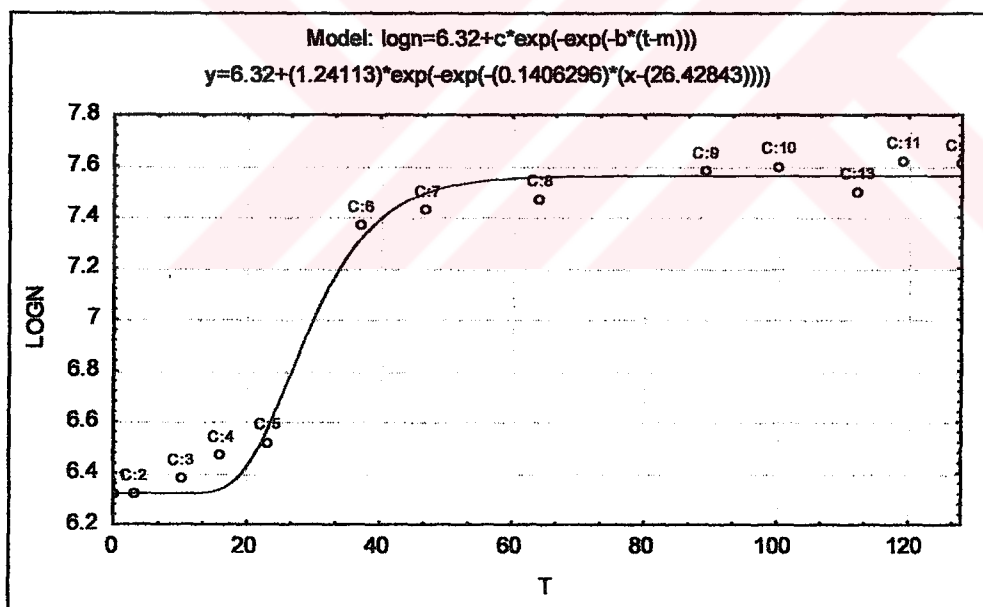


Figure B. 74. Growth curve of *B. cereus* at 32°C, 7.3 pH, 342 mM NaCl and 200 mM sodium lactate.

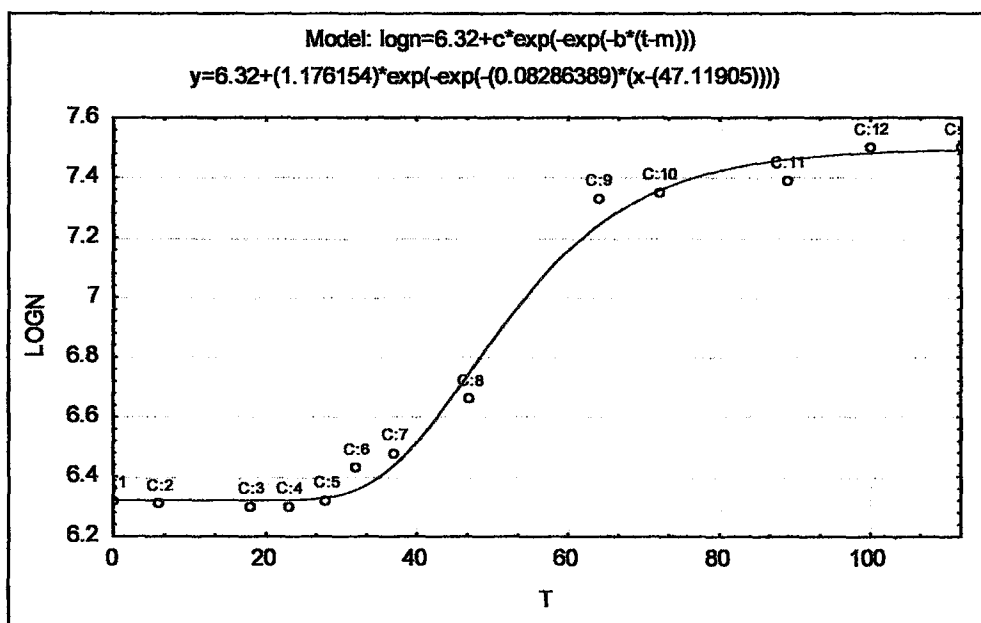


Figure B. 75. Growth curve of *B. cereus* at 32°C, 7.3 pH, 342 mM NaCl and 400 mM sodium lactate.

APPENDIX C. STATISTICAL ANALYSES RESULTS OF SECONDARY LEVEL MODELS

Table C. 1. Polynomial Model for Lag Time (LAG)

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercept			285.228	112.347	2.53881	0.01306
T	-3.2785	0.52567	-11.077	1.77607	-6.2368	2E-08
PH	-1.1776	0.80371	-50.833	34.6942	-1.4652	0.14679
NAL	3.03949	0.51595	0.51645	0.08767	5.89109	8.7E-08
NACL	1.27968	0.40697	0.15839	0.05037	3.1444	0.00234
T2	2.89988	0.2695	0.22623	0.02102	10.7602	3.3E-17
PH2	1.31509	0.81285	4.38997	2.71344	1.61786	0.10963
NAL2	0.40429	0.10898	0.00014	3.8E-05	3.70988	0.00038
NACL2	-0.3457	0.21054	-6E-05	3.9E-05	-1.642	0.10452
TPH	-0.2205	0.389	-0.1084	0.19128	-0.5667	0.5725
TNAL	-0.466	0.12476	-0.003	0.00079	-3.7352	0.00035
TNACL	-0.2797	0.1215	-0.0012	0.00054	-2.3023	0.02392
PHNAL	-2.7444	0.56842	-0.066	0.01367	-4.8282	6.5E-06
PHNACL	-0.4577	0.35722	-0.0084	0.00653	-1.2812	0.20382
NALNACL	0.27723	0.06336	0.00014	3.1E-05	4.37562	3.6E-05
R= 0.96244386 R ² = 0.92629818 Adjusted R ² = .91340036						
F(14.80)=71.818 p<.00000 Std. Err. Est.: 8.1176						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	66254.8	14	4732.48	71.8182	3E-39	
Residual	5271.63	80	65.8953			
Total	71526.4					

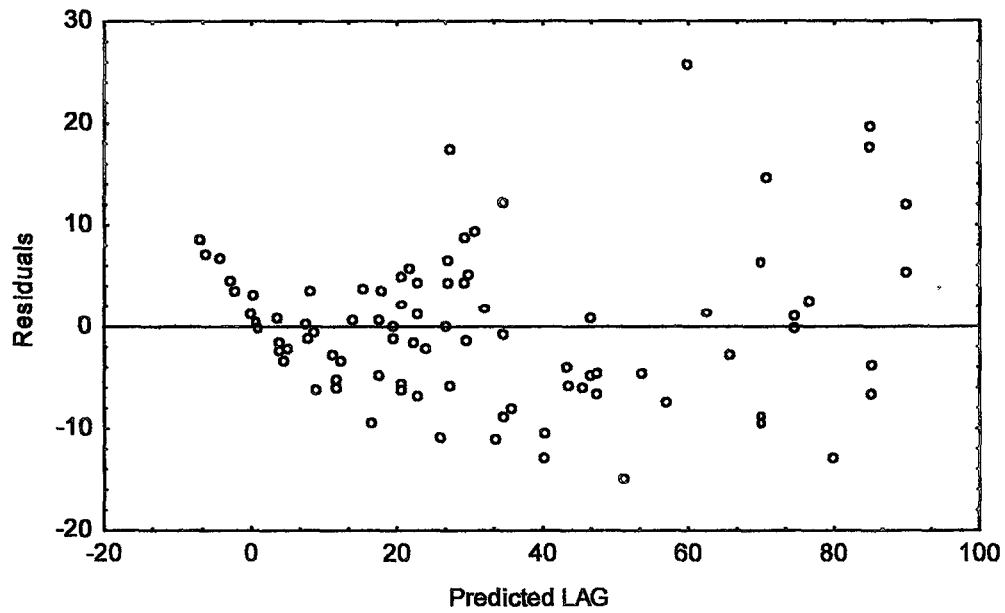


Figure C. 1. Predicted versus residual values for the LAG model.

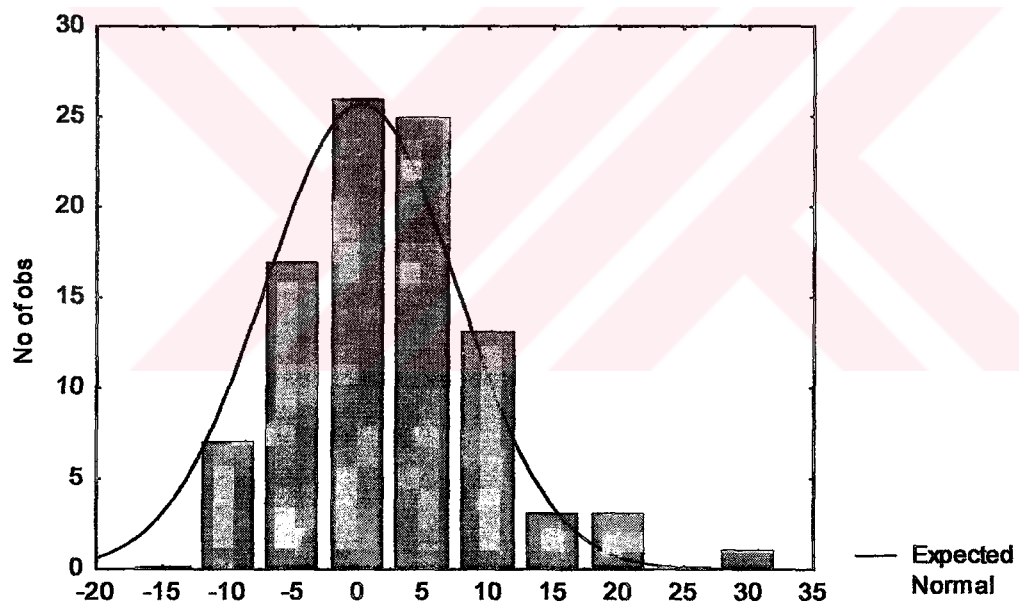


Figure C. 2. Distribution of residuals for the LAG model.

Table C. 2. Polynomial Model for the Natural Logarithm of Lag Time (ln LAG)

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			19.702	4.44532	4.43207	2.9E-05
T	-1.9576	0.4112	-0.3346	0.07027	-4.7607	8.4E-06
PH	-1.7571	0.62869	-3.8368	1.37277	-2.7949	0.0065
NAL	1.73859	0.40359	0.01494	0.00347	4.30781	4.6E-05
NACL	0.43574	0.31834	0.00273	0.00199	1.36876	0.17491
T2	1.02836	0.21081	0.00406	0.00083	4.87806	5.4E-06
PH2	1.67158	0.63584	0.28225	0.10736	2.62893	0.01027
NAL2	0.08302	0.08525	1.5E-06	1.5E-06	0.97395	0.33302
NACL2	-0.75	0.16469	-7E-06	1.5E-06	-4.5539	1.9E-05
TPH	-0.2625	0.30429	-0.0065	0.00757	-0.8626	0.39093
TNAL	0.62103	0.09759	0.0002	3.1E-05	6.36334	1.2E-08
TNACL	0.79832	0.09504	0.00018	2.1E-05	8.39953	1.3E-12
PHNAL	-1.7389	0.44464	-0.0021	0.00054	-3.9109	0.00019
PHNACL	0.19193	0.27943	0.00018	0.00026	0.68688	0.49415
NALNACL	-0.0659	0.04956	-2E-06	1.2E-06	-1.3293	0.18753
R= 0.97719131		R ² = 0.95490285		Adjusted R ² = 0.94701085		
F(14.80)= 121.00		p<.00000		Std. Err. Est.: 0.32119		
Analysis of Variance						
	Sums of Squares	df	Mean Squares		F	p-level
Regress.	174.758	14	12.4827		120.996	0
Residual	8.25329	80	0.10317			
Total	183.011					

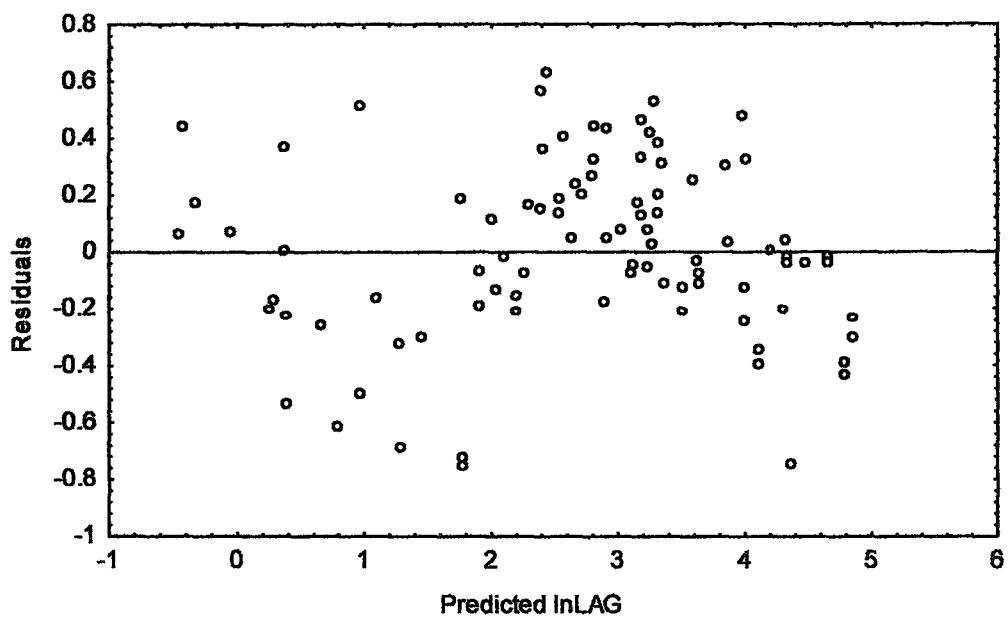


Figure C. 3. Predicted versus residual values for the ln LAG model.

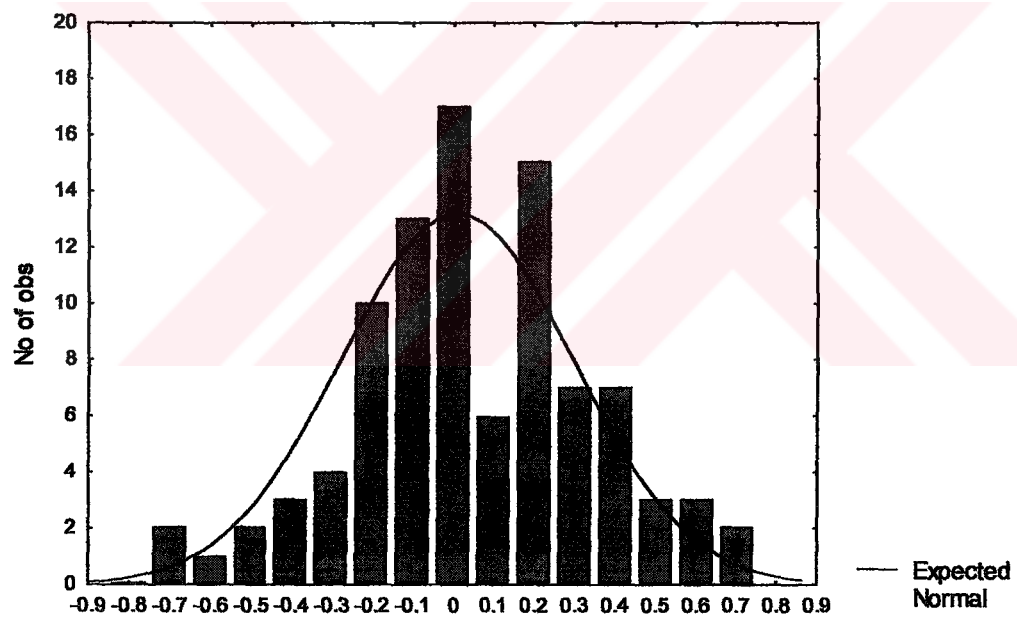


Figure C. 4. Distribution of residuals for the ln LAG model.

Table C. 3. Polynomial Model for the Natural Logarithm of Lag Time (ln LAG) only with Significant ($P < 0.05$) Coefficients

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			21.4622	4.20534	5.10357	2E-06
T	-2.2698	0.18693	-0.3879	0.03195	-12.143	3.5E-20
PH	-1.9333	0.61887	-4.2215	1.35133	-3.124	0.00245
NAL	1.6658	0.37382	0.01432	0.00321	4.45618	2.6E-05
NACL	0.54353	0.17314	0.0034	0.00108	3.13927	0.00234
T2	1.10131	0.19381	0.00435	0.00076	5.68251	1.9E-07
PH2	1.79539	0.63214	0.30316	0.10674	2.84018	0.00565
NACL2	-0.7002	0.16098	-6E-06	1.5E-06	-4.3498	3.8E-05
TNAL	0.58921	0.09005	0.00019	2.9E-05	6.54324	4.5E-09
TNACL	0.77739	0.0949	0.00018	2.1E-05	8.19164	2.5E-12
PHNAL	-1.6006	0.37783	-0.0019	0.00046	-4.2364	5.8E-05
R= 0. 97552305 R ² = 0. 95164523 Adjusted R ² = 0. 94588870						
F(14.80)= 165.32 p<.00000 Std. Err. Est.: 0. 32458						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	174.162	10	17.4162	165.316	0	
Residual	8.84947	84	0.10535			
Total	183.011					

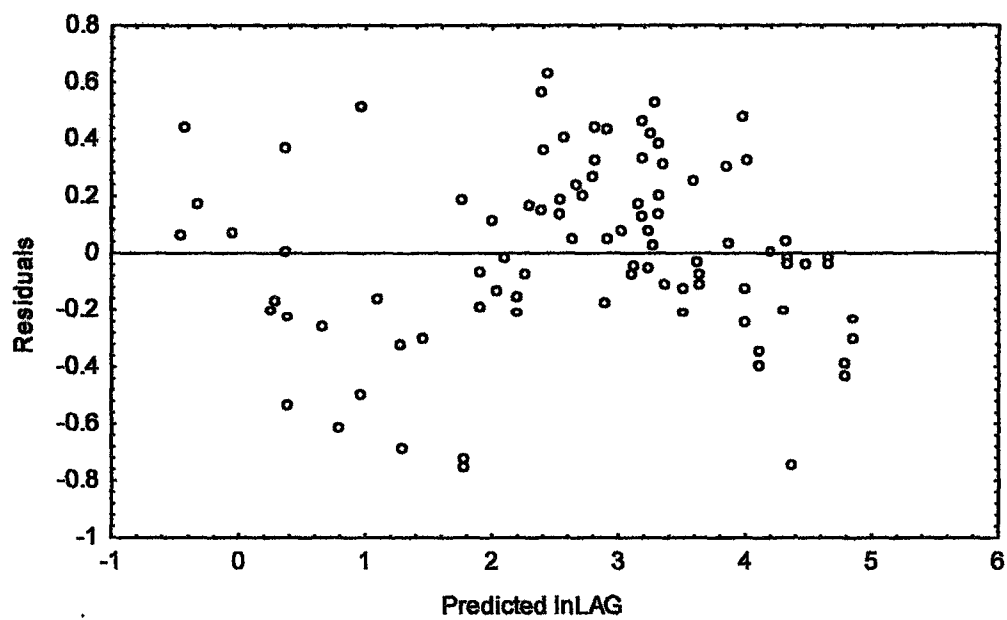


Figure C. 5. Predicted versus residual values for the reduced ln LAG model.

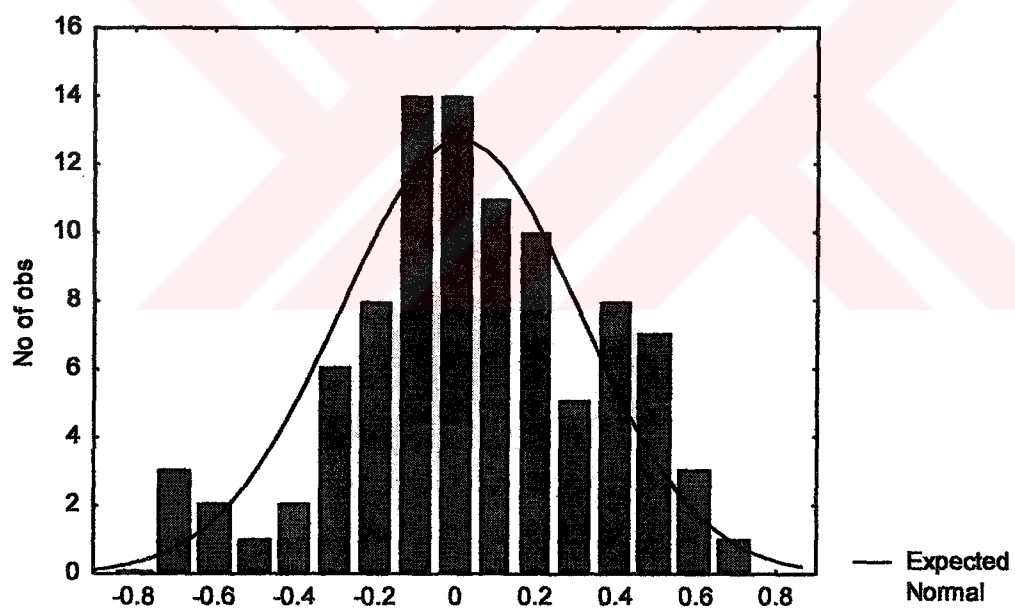


Figure C. 6. Distribution of residuals for the reduced ln LAG model.

Table C. 4. Modified Arrhenius-Davey Model for the Natural Logarithm of Lag Time (ln (1/LAG))

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			-538.69	90.5073	-5.9519	5.6E-08
PH	0.40036	0.80656	0.87421	1.76115	0.49639	0.62089
NAL	-0.5972	0.10209	-0.0051	0.00088	-5.8502	8.7E-08
NACL	-1.2365	0.24584	-0.0077	0.00154	-5.0298	2.7E-06
PH2	-0.183	0.8078	-0.0309	0.1364	-0.2266	0.82131
NAL2	-0.0305	0.10193	-5E-07	1.8E-06	-0.2991	0.7656
NACL2	0.70944	0.24405	6.6E-06	2.3E-06	2.90693	0.00464
Z	22.0181	3.61158	326064	53483.7	6.09651	3E-08
Z2	-22.829	3.61527	-5E+07	7873643	-6.3147	1.1E-08
R= 0. 94275947 R²= 0. 88879541 Adjusted R²= 0. 87845080						
F(8,86)= 85.919 p<.00000 Std. Err. Est.: 0. 48646						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	162.66	8	20.3324	85.9187	1E-37	
Residual	20.3517	86	0.23665			
Total	183.011					

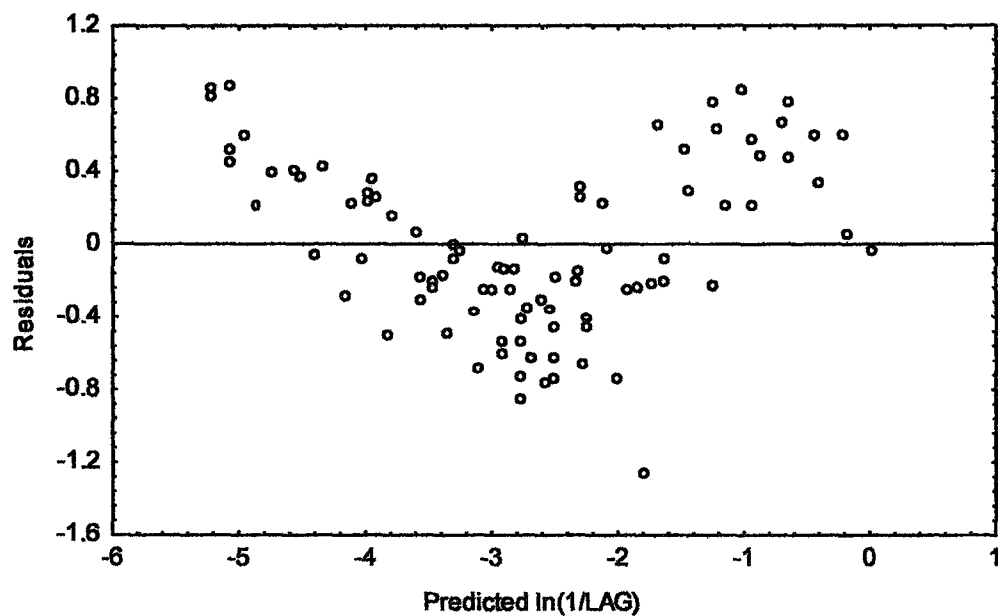


Figure C. 7. Predicted versus residual values for the $\ln(1/\text{LAG})$ (modified Arrhenius-Davey) model.

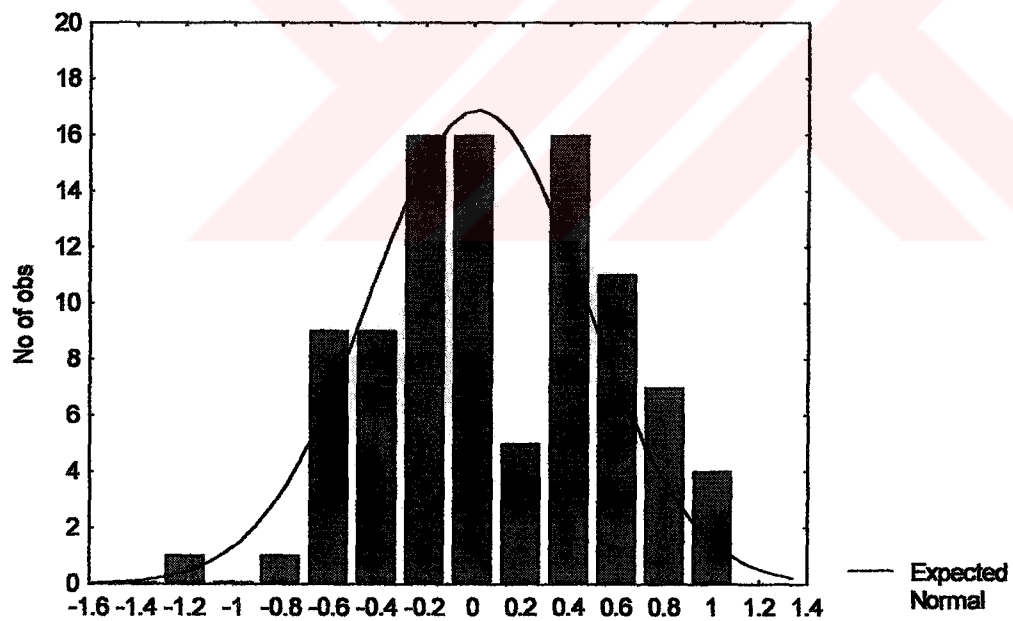


Figure C. 8. Distribution of residuals for the $\ln(1/\text{LAG})$ (modified Arrhenius-Davey) model.

Table C. 5. Polynomial Model for Growth Rate (GR)

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			-2.0921	0.81796	-2.5577	0.01243
T	0.88745	0.64212	0.01787	0.01293	1.38205	0.1708
PH	1.96032	0.98176	0.50437	0.2526	1.99675	0.04925
NAL	-0.1676	0.63024	-0.0002	0.00064	-0.266	0.79093
NACL	1.57741	0.49713	0.00116	0.00037	3.17307	0.00214
T2	0.08517	0.3292	4E-05	0.00015	0.2587	0.79653
PH2	-1.5949	0.99293	-0.0317	0.01976	-1.6063	0.11216
NAL2	0.57156	0.13312	1.2E-06	2.8E-07	4.2936	4.9E-05
NACL2	0.64478	0.25718	7E-07	2.8E-07	2.50713	0.0142
TPH	0.36556	0.47517	0.00107	0.00139	0.76932	0.44397
TNAL	-1.3867	0.1524	-5E-05	5.8E-06	-9.0991	5.6E-14
TNACL	-1.0227	0.14842	-3E-05	3.9E-06	-6.8907	1.1E-09
PHNAL	-0.0719	0.69434	-1E-05	1E-04	-0.1035	0.91783
PHNACL	-2.0216	0.43635	-0.0002	4.8E-05	-4.633	1.4E-05
NALNACL	0.43578	0.07739	1.3E-06	2.3E-07	5.63068	2.6E-07
R= 0. 94341246 R²= 0. 89002707 Adjusted R²= 0. 87078181						
F(14.80)=46.24 p<.00000 Std. Err. Est.: 0. 05910						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	2.26154	14	0.16154	46.2466	2.1E-32	
Residual	0.27944	80	0.00349			
Total	2.54098					

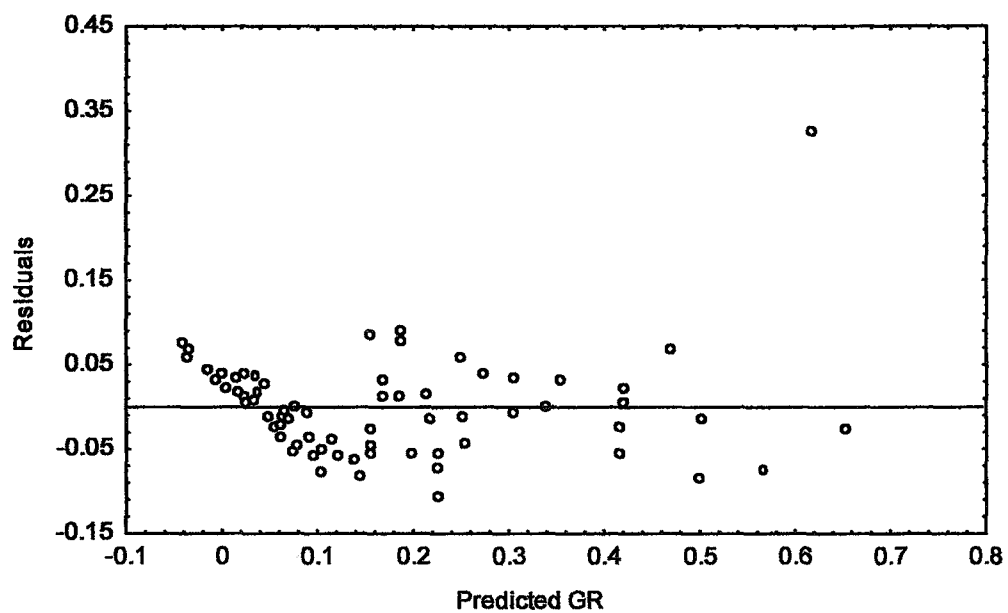


Figure C. 9. Predicted versus residual values for the GR model.

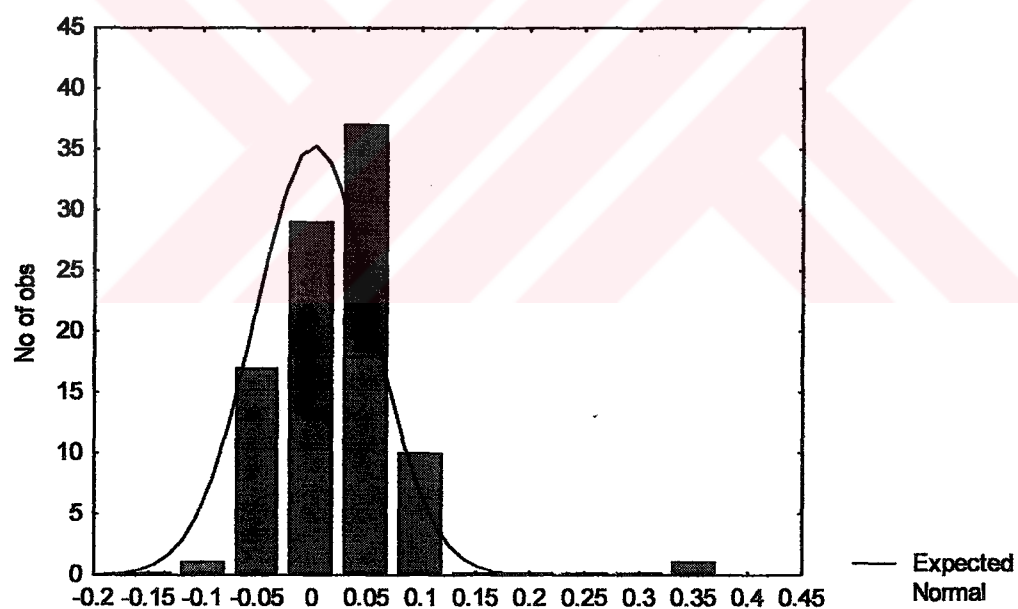


Figure C. 10. Distribution of residuals for the GR model.

Table C. 6. Polynomial Model for Natural Logarithm of Growth Rate (ln GR)

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			-19.981	3.70928	-5.3867	7E-07
T	3.30204	0.47384	0.40864	0.05864	6.96874	8.1E-10
PH	2.2691	0.72446	3.58777	1.14547	3.13214	0.00242
NAL	-1.4731	0.46507	-0.0092	0.00289	-3.1675	0.00218
NACL	0.43235	0.36684	0.00196	0.00166	1.17858	0.24206
T2	-1.2108	0.24293	-0.0035	0.00069	-4.9842	3.5E-06
PH2	-1.6853	0.7327	-0.2061	0.08959	-2.3002	0.02404
NAL2	0.01766	0.09823	2.3E-07	1.3E-06	0.17978	0.85778
NACL2	0.80949	0.18978	5.4E-06	1.3E-06	4.26549	5.4E-05
TPH	-1.0711	0.35064	-0.0193	0.00632	-3.0547	0.00306
TNAL	-0.681	0.11246	-0.0002	2.6E-05	-6.0555	4.3E-08
TNACL	-0.3776	0.10952	-6E-05	1.8E-05	-3.4475	0.0009
PHNAL	1.3283	0.51237	0.00117	0.00045	2.59247	0.01133
PHNACL	-1.354	0.32199	-0.0009	0.00022	-4.205	6.8E-05
NALNACL	0.05127	0.05711	9.3E-07	1E-06	0.89767	0.37205
R= 0. 96959620 R²= 0. 94011679 Adjusted R²= 0. 92963723						
F(14.80)=89.710 p<.00000 Std. Err. Est.: 0. 26801						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	90.2144	14	6.44388	89.7096	8.1E-43	
Residual	5.74644	80	0.07183			
Total	95.9608					

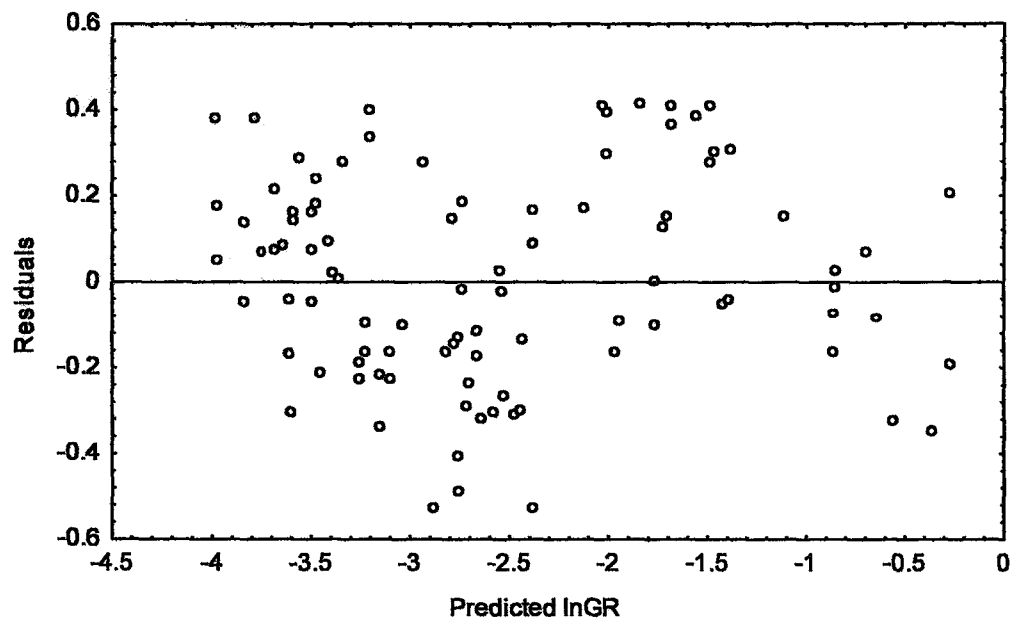


Figure C. 11. Predicted versus residual values for the ln GR model.

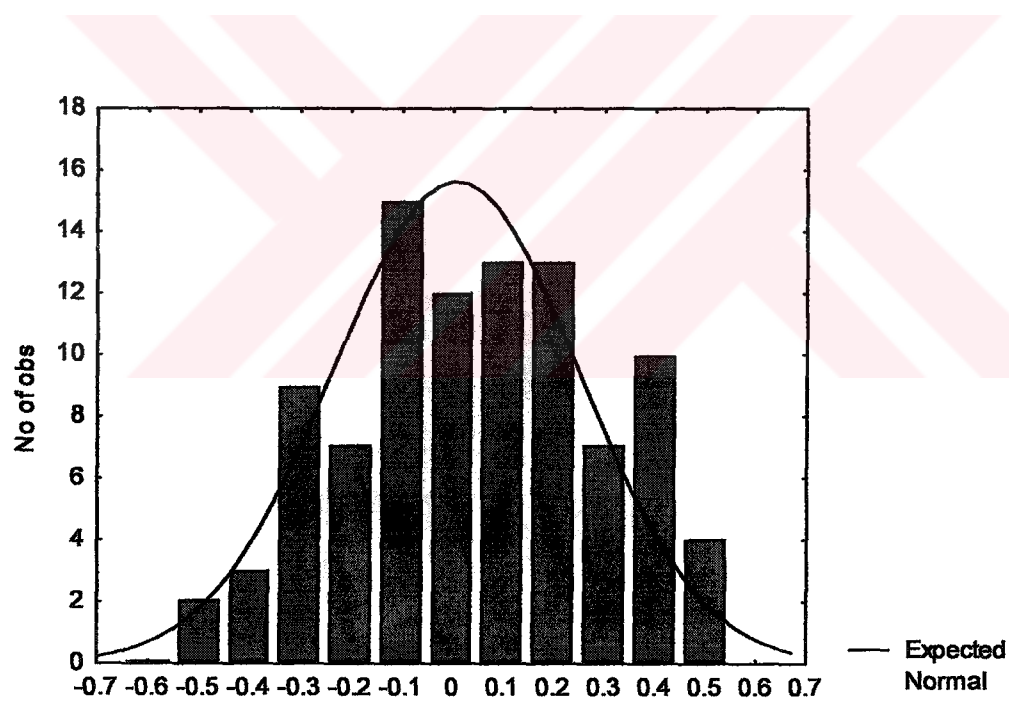


Figure C. 12. Distribution of residuals for the ln GR model.

Table C. 7. Polynomial Model for Natural Logarithm of Growth Rate (ln GR) only with Significant ($P < 0.05$) Coefficients

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			-20.251	3.67882	-5.5047	4E-07
T	3.3212	0.46676	0.41101	0.05776	7.11547	3.6E-10
PH	2.36762	0.71619	3.74355	1.13239	3.30588	0.0014
NAL	-1.5356	0.42493	-0.0096	0.00264	-3.6137	0.00052
T2	-1.1948	0.24109	-0.0034	0.00069	-4.9558	3.8E-06
PH2	-1.8154	0.72199	-0.222	0.08828	-2.5145	0.01385
NACL2	0.89932	0.15659	6E-06	1.1E-06	5.74327	1.5E-07
TPH	-1.1248	0.34059	-0.0203	0.00613	-3.3024	0.00142
TNAL	-0.661	0.10805	-0.0002	2.5E-05	-6.1173	3E-08
TNACL	-0.3556	0.1071	-6E-05	1.7E-05	-3.32	0.00134
PHNAL	1.42719	0.43015	0.00126	0.00038	3.31787	0.00135
PHNACL	-0.9915	0.16753	-0.0007	0.00011	-5.9184	7E-08
R= 0. 96884489 R ² = 0. 93866041 Adjusted R ² = 0. 93053107						
F(11.83)=115.47 p<.00000 Std. Err. Est.: 0. 26630						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	90.0746	11	8.1886	115.466	1.4E-45	
Residual	5.8862	83	0.07092			
Total	95.9608					

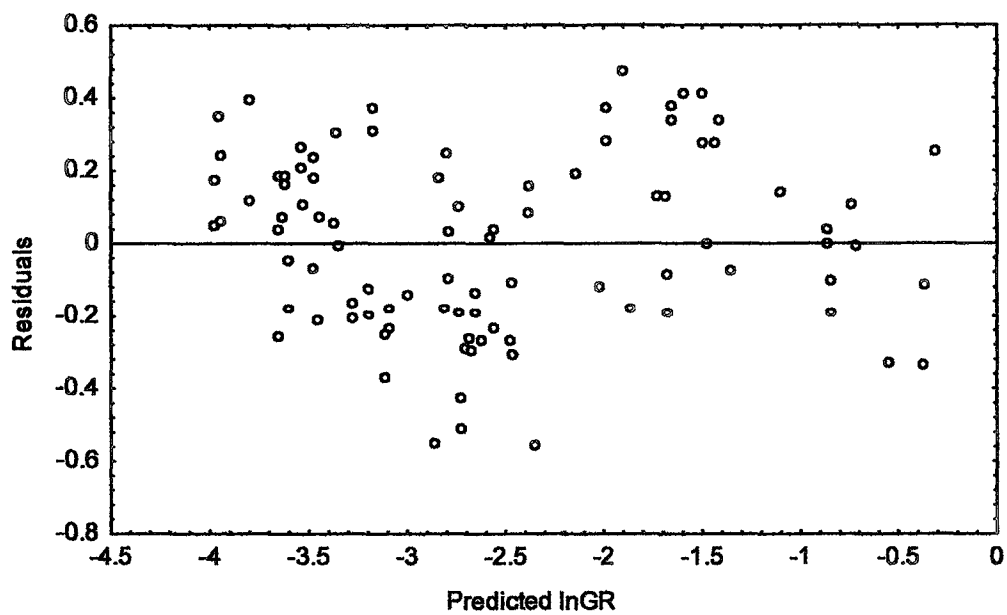


Figure C. 13. Predicted versus residual values for the reduced ln GR model.

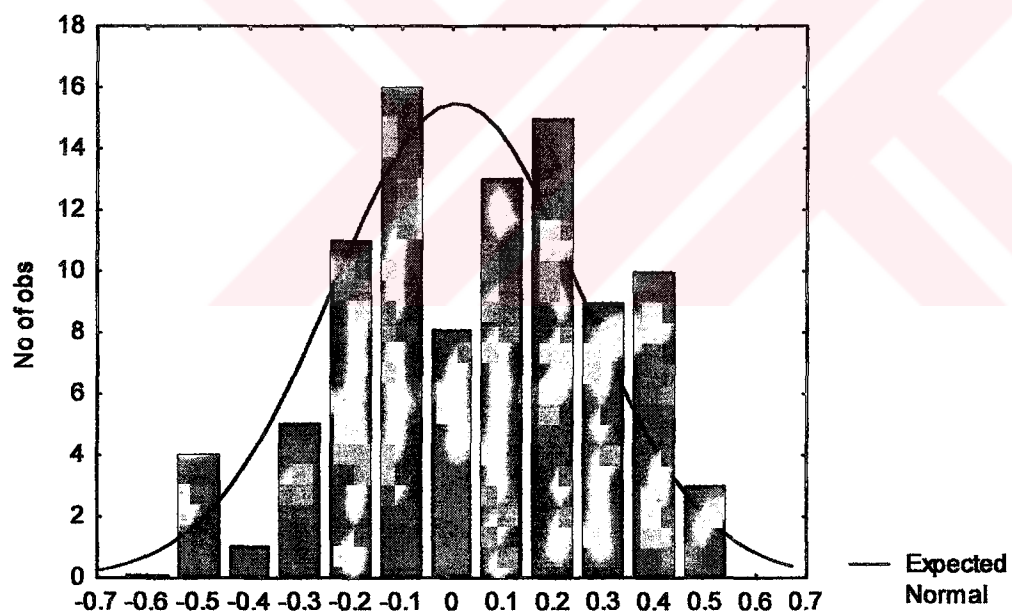


Figure C. 14. Distribution of residuals for the reduced ln GR model.

Table C. 8. Modified Arrhenius-Davey Model for the Natural Logarithm of Growth Rate (ln GR)

	BETA	St. Err. BETA	B	St. Err. B	t(80)	p-level
Intercpt			-310.92	75.1981	-4.1346	8.2E-05
PH	0.6865	0.92544	1.08545	1.46326	0.7418	0.46023
PH2	-0.4825	0.92687	-0.059	0.11333	-0.5206	0.604
NAL	-0.7893	0.11713	-0.0049	0.00073	-6.7383	1.7E-09
NAL2	0.07145	0.11695	9.2E-07	1.5E-06	0.61091	0.54287
NACL	-1.2189	0.28208	-0.0055	0.00128	-4.3211	4.2E-05
NACL2	0.84068	0.28003	5.6E-06	1.9E-06	3.00217	0.00351
Z	17.5173	4.14393	187844	44436.9	4.2272	5.9E-05
Z2	-18.278	4.14816	-3E+07	6541823	-4.4063	3E-05
R= 0. 92390263 R²= 0. 8535960 Adjusted R²= 0. 83997710						
F(8.86)=62.677 p<.00000 Std. Err. Est.: 0. 40418						
Analysis of Variance						
	Sums of Squares	df	Mean Squares	F	p-level	
Regress.	81.9118	8	10.239	62.677	1.3E-32	
Residual	14.049	86	0.16336			
Total	95.9608					

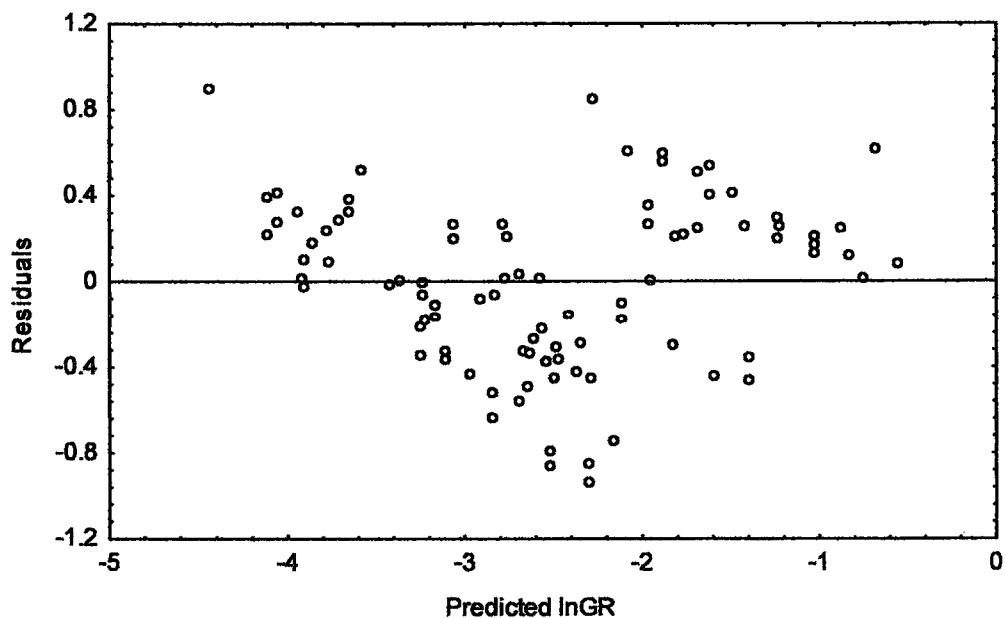


Figure C. 15. Predicted versus residual values for the ln GR (modified Arrhenius-Davey) model.

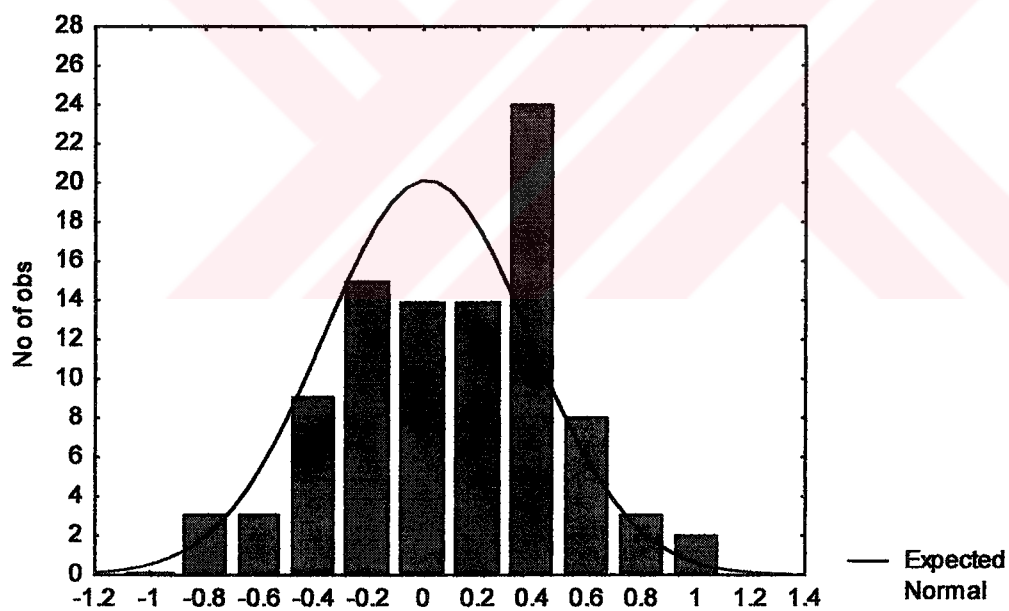


Figure C. 16. Distribution of residuals for the ln GR (modified Arrhenius-Davey) model.

APPENDIX D. GROWTH CURVES OF *B. CEREUS* IN MILK AND MEAT BROTH

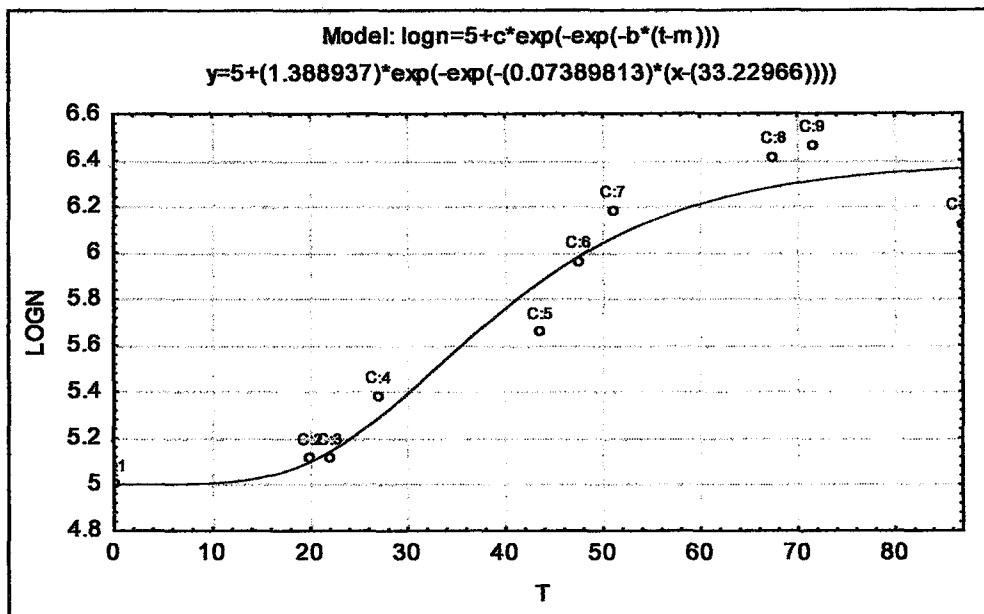


Figure D. 1. Growth curve of *B. cereus* in milk at 12°C.

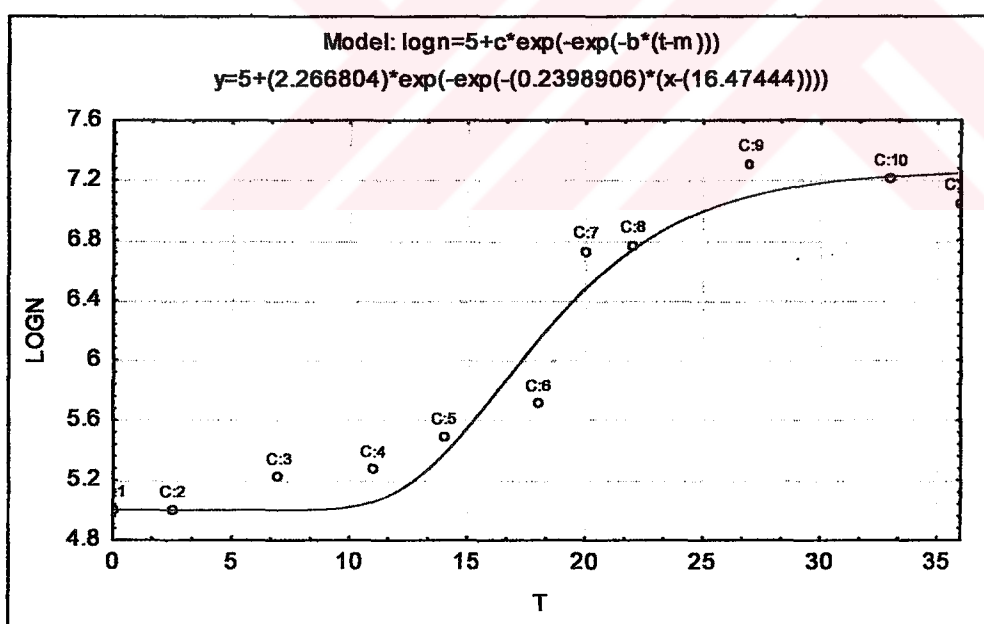


Figure D. 2. Growth curve of *B. cereus* in milk at 18°C.

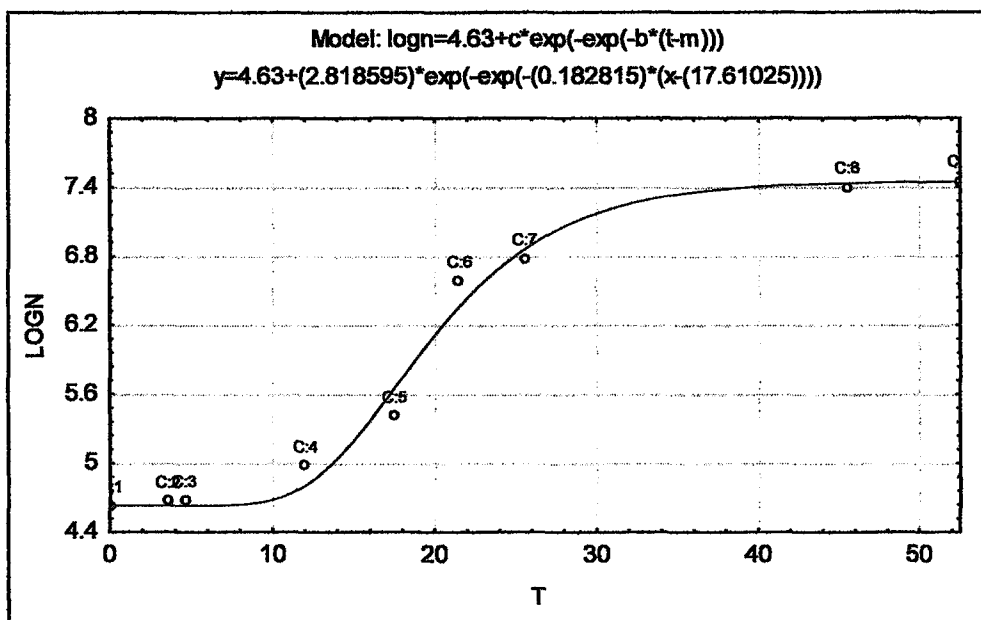


Figure D. 3. Growth curve of *B. cereus* in meat broth containing 85 mM NaCl at 18°C.

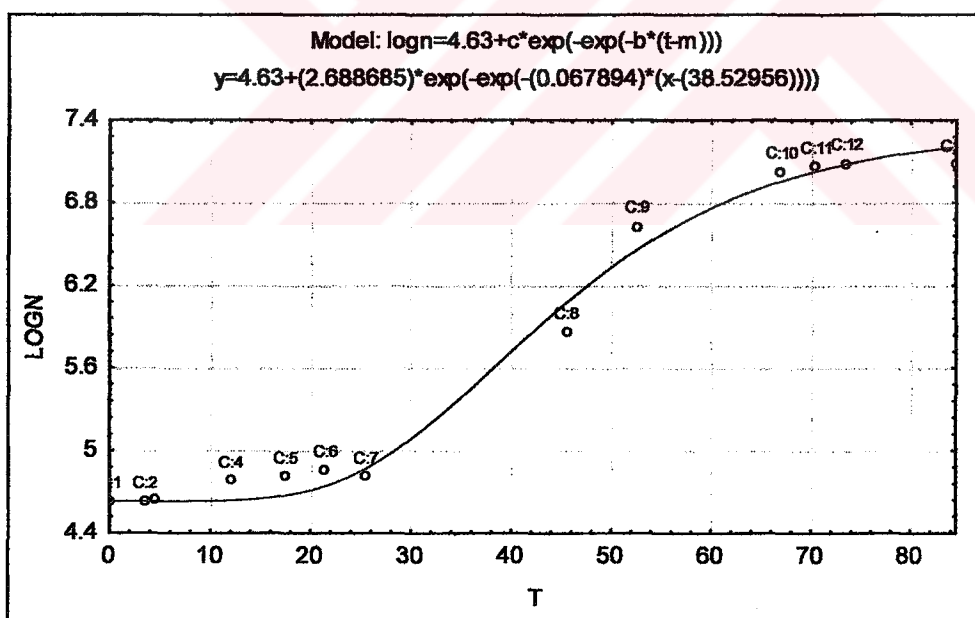


Figure D. 4. Growth curve of *B. cereus* in meat broth containing 85 mM NaCl and 200 mM sodium lactate at 18°C.

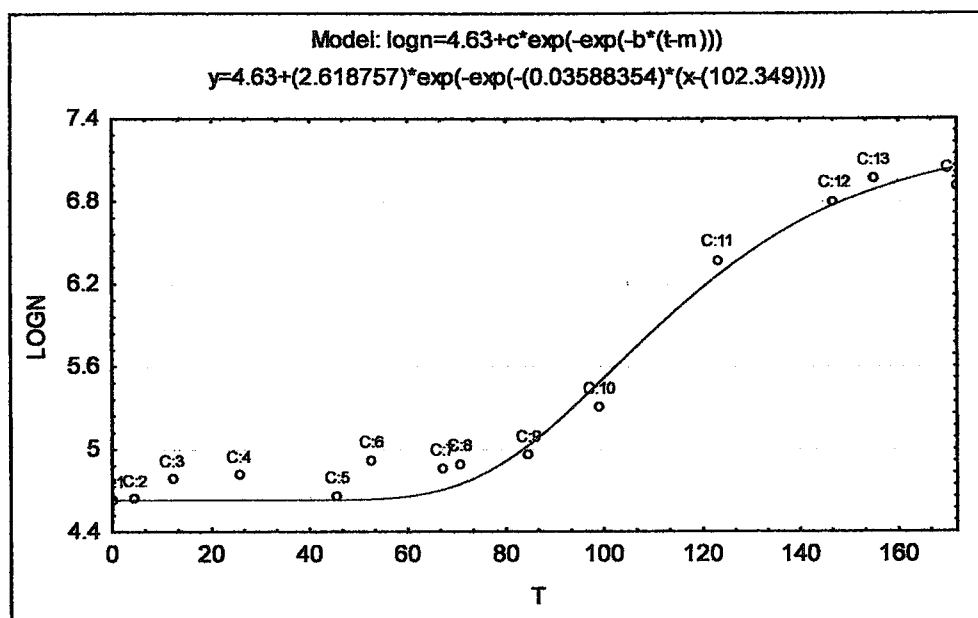


Figure D. 5. Growth curve of *B. cereus* in meat broth containing 85 mM NaCl and 400 mM sodium lactate at 18°C.

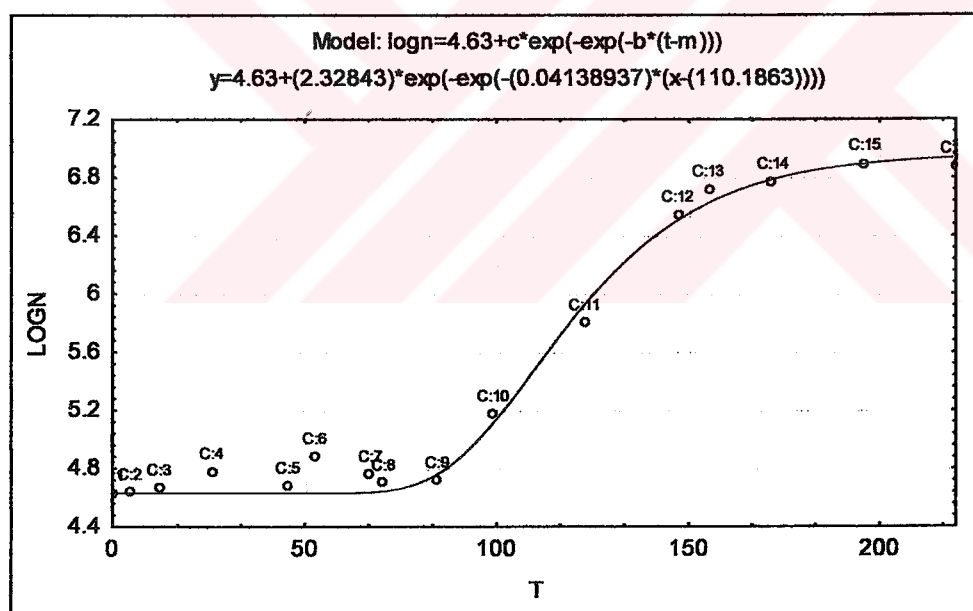


Figure D. 6. Growth curve of *B. cereus* in meat broth containing 172 mM NaCl and 400 mM sodium lactate at 18°C.

VITA

Hülya Ölmez was born on December 5, 1972 in Ankara, Turkey. She received her Bachelor's and Master's degrees from the Department of Food Engineering, Middle East Technical University, Ankara, Turkey in June 1994 and June 1996, respectively. She then began to work in the Food Science and Technology Research Institute, The Scientific and Technical Research Council of Turkey, Marmara Research Center in 1996. She is still working as a researcher in this Institute.

