Journal of Industrial Microbiology, 12 (1993) 286–290

Published by The Macmillan Press Ltd

Conductance measurements for data generation in predictive modeling

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(Received 22 September 1992)

Key words: Conductance; Predictive modeling; *Yersinia enterocolitica*

SUMMARY

The electrical resistance of a growth medium inoculated with bacteria may be automatically recorded throughout an incubation period without the necessity for sampling. The rate of change in conductance is dependent on the bacteria studied, the medium composition and the prevailing growth conditions.

The effect of growth medium composition, growth conditions and inoculum level on the conductance response was studied for *Yersinia enterocolitica* O:3. A large number of combinations of factors affecting the growth/activity of the bacteria could be studied simultaneously due to the large instrumental capacity of the Malthus 2000. A polynomial model based on conductance measurements was developed for *Y. enterocolitica* describing the effect of temperature, pH and L-lactate level on conductance response curve parameters. The model was used for predicting growth rates. Growth rates calculated from bacterial counts of *Y. enterocolitica* growing in minced pork corresponded to growth rates predicted using the polynomial conductance models.

INTRODUCTION

By fitting bacterial growth curves with mathematical equations the specific growth rate can be calculated from the curve parameters. A large number of data points is essential in order to get precise determinations of specific growth rates and to generate good predictive models for bacterial growth [3]. Viable count determinations have been used as data when constructing this type of model [6,7]. Since viable count determinations are laborious there is a need for simpler and more automated techniques. In order to model the response of bacteria to environmental factors, estimates such as reciprocal time to reach a specific absorbance value [1,8] or increase in absorbance during a fixed incubation time [4] have been used.

Metabolically active bacteria suspended in a suitable medium change the electrical resistance of the growth medium [5]. The change can be electrically monitored using electrodes. Commercial systems exist that automatically record the change in conductance throughout the incubation period. In the Malthus 2000 bacteria are suspended in a suitable medium and dispended to a cell consisting of a glass tube and a cap with a pair of electrodes. A large number of samples may be run simultaneously.

Conductance is a measurement of the current flow that results upon the application of an electrical force and involves the migration of charge compounds/ions. The conductance of a solution is dependent upon the number of charged compounds it contains, their relative concentration and inherent mobility. Conductance changes in the presence of microrganisms arise from the production and utilization of charged compounds and are correlated directly to the activity of the cells, but not necessarily to bacterial growth [5].

In the present paper, the conductance response of *Y. enterocolitica* O:3 was studied. A polynomial model based on conductance measurements of *Y. enterocolitica* describing the effect of temperature, pH and L-lactate level on conductance response curve parameters was developed and validated with growth in pork.

MATERIAL AND METHODS

The conductance response of *Yersinia enterocolitica* O:3 was monitored on a Malthus 2000 (Malthus Instruments Ltd., Crawley, UK). The medium used contained 3.25% SPYE (special peptone yeast extract; Malthus Instruments Ltd.) and 5% skim milk.

A cocktail of three strains of *Y. enterocolitica* O:3 was used as an inoculum. The inoculum was prepared in a standardized way starting from pure cultures stored at −70 °C. Cultivation was performed in two steps, 24 h at 28 °C and 24 h at 15 °C. After mixing the three strains (1:1:1), a cocktail was obtained. To 2 ml of medium, 0.02 ml of cocktail was added.

The conductance response curve parameters were calculated by fitting a curve to the conductance data using the Gompertz equation [6]. The Gompertz curve is given by $L(t) = A + C \exp(-\exp(-B(t-M)))$. The A parameter corresponds to the asymptotic initial conductance value, C is the asymptotic maximal conductance value, B is the relative rate of conductance change at M, where M is the

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time when the absolute conductance change is at maximum. Calculations were made using SYSTAT (version 5.0; SYSTAT Inc., 1990).

Viable count determinations were made from Malthus cells during incubation of *Y. enterocolitica* at pH 6.2 and 18 °C, by plating on Tryptone Glucose Extract agar (TGE; Oxoid, Basingstoke, UK). The results were correlated to conductance values in the corresponding cells.

The effect of inoculum level on *A*, *B*, *C* and *M* parameters was studied at pH 5.4 and pH 6.2. The inoculum levels were 10^3, 10^5 and 10^7 cfu ml^{-1}.

A polynomial model based on conductance data describing the effect of pH value (5.4, 5.9, 6.2, 6.5), temperature (7, 12, 15, 18, 23 °C) and L-lactate concentration (0, 0.4, 0.8, 1.2%) on the *A*, *B*, *C* and *M* Gompertz parameters was developed for *Y. enterocolitica*. The inoculum level used was 10^7 cfu ml^{-1}. All combinations were run in triplicates.

The levels of CO_2 and O_2 in a Malthus cell were monitored during incubation of *Y. enterocolitica* at pH 6.2 and 18 °C. Gas samples of 0.2 ml were taken from the closed Malthus cell through the membrane with a gas-tight syringe and analyzed for CO_2 and O_2 content with a gas chromatograph (Varian 3700) fitted with a thermal conductivity detector and a parallel system of MS 5A (Applied Science, Penna, USA) and Porapak Q (Waters Associates Inc., Milford, USA) columns. The temperature was 50 °C.

A pork loin was sterilized by burning the surface with a gas-burner. After cutting away the burnt meat, mincing was performed and samples were taken out for determination of the L-lactate concentration [2] and pH value. Four experiments were carried out using four pork loins. The pH values of the pork loins were pH 5.4, 5.7, 6.1 and 6.7; the corresponding L-lactate concentrations were 9.16, 8.74, 6.96 and 3.98 mg g^{-1}, respectively. To the sterilized minced pork meat, *Y. enterocolitica* was added to a level of about 10^6 cfu ml^{-1}. The inoculated meat was stored in Petri dishes with a lid and wrapped with oxygen permeable film in order to prevent drying. Storage was performed at 18 °C under aerobic conditions. During storage, the growth of *Y. enterocolitica* was monitored by plating on TGE agar. The maximum specific growth rate was calculated as the maximum slope of the plot $\log_e$ (viable count) versus time. Each growth curve was based on 24 bacterial count determinations.

RESULTS AND DISCUSSION

Conductance response curve

Typical conductance response curves of *Y. enterocolitica* are shown in Fig. 1a. The conductance curve initially consisted of an inactive stage when there was no significant increase in conductance. The inoculum number of *Y. enterocolitica* and the detection time (time up until a significant increase in conductance) were inversely related (Fig.1b). The lower the inoculum number of bacteria the longer the time until detection. This basic principle is used when quantative determinations are made using conductance analysis [5].

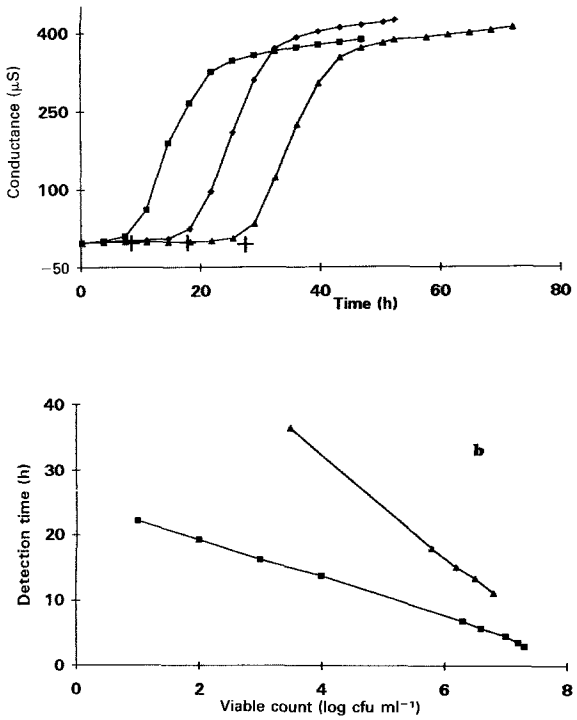


Fig. 1. (a) Change in conductance during growth of *Y. enterocolitica* O:3 in SPYE-skim milk medium using three inoculum levels: 10^3 cfu ml^{-1} (▲), 10^5 cfu ml^{-1} (◆), and 10^7 cfu ml^{-1} (■); at pH 6.2 and 18 °C. Detection time is marked with +. (b) Relationship between detection time and logarithm of inoculum number at an incubation temperature of 15 °C, pH 6.0 (▲) or 25 °C, pH 6.0 (■).

The growth conditions prevailing during incubation in the cell affected the conductance response curve of *Y. enterocolitica* in three ways. The detection time, the slope of the curve and the maximal response were affected (Fig. 2). When altering the growth conditions from favorable to less favorable, the detection time increased, the rate of the conductance change decreased and the response decreased.

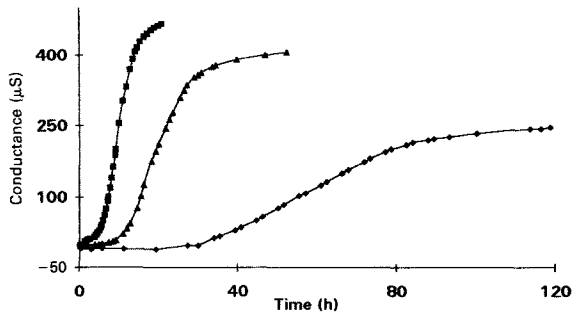


Fig. 2. Change in conductance during growth of *Y. enterocolitica* O:3 at, 23 °C (■), 15 °C (▲) and 7 °C (◆) in SPYE-skim milk medium at pH 6.2.

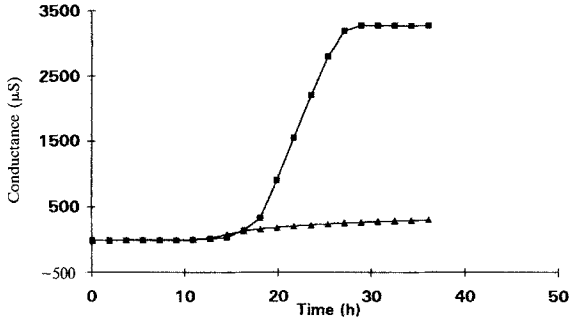


Fig. 3. Change in conductance during growth of *Y. enterocolitica* O:3 at 25 °C, pH 6.0 in SPYE-skim milk medium with 0% urea (▲) or 0.2% urea (■).

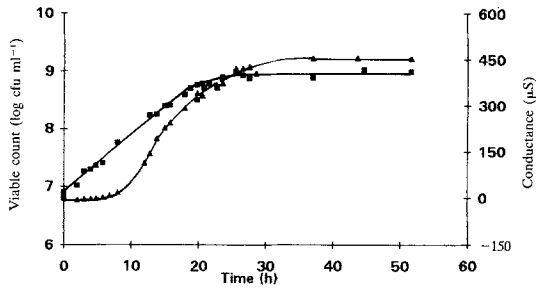


Fig. 5. Viable count (■) and change (▲) in conductance during growth of *Y. enterocolitica* O:3 at 18 °C, pH 6.2 in SPYE-skim milk medium.

Growth medium

The biochemical changes taking place in a growth medium during bacterial growth are complex in nature and are difficult to predict. For the yeast *Rhodotorula*, conductance either increased or decreased with growth in media with different nitrogen sources [10]. Analyses of the medium showed an increase in ammonium ion and aspartate concentration, while L-asparagine decreased.

The addition of urea to a growth medium inoculated with *Y. enterocolitica* gave a very high response (Fig. 3). However, we have decided not to use a response enforcing substrate, such as urea, in the medium when constructing kinetic growth models. In this case, it is essential that the response does not reflect a specific enzymatic activity, e.g. urease, since this would presumably lead to a predictive model for the enzyme activity, as affected by environmental factors.

The charge-carrying capacity of the medium is important. Since amino acids and peptides are active charge carriers, the peptide and protein components of the media are important for the conductance response [5]. By adding skim milk to the medium for *Yersinia*, the response was increased (Fig. 4). During good incubation conditions, a response of about 500 µs was attained for *Y. enterocolitica*.

The change in conductance can largely be masked by high salt concentrations. Thus, the amount of NaCl that may be included in the medium is limited. A high salt concentration will give too high an initial conductance value and the measuring area of the instrument will be overtraced. The maximal NaCl concentration that may be applied is, in accordance with our experience, 3%, using the normal cells and software supplied with the Malthus 2000. However, the use of indirect conductimetry may solve this problem. In indirect conductimetry, the electrodes are not placed in direct contact with the bacteria-medium mixture [11].

Conductance and viable count

The shape of the conductance curve with time resembles that of a conventional bacterial growth curve. However, the conductance response curve and the bacterial growth curve of *Y. enterocolitica* were not totally synchronous (Fig. 5). The conductance curve was delayed at the start due to the detection threshold of the instrument.

The log viable count versus conductance, during the active stage of the conductance curve for *Y. enterocolitica* is shown in Fig. 6. From the figure it may be seen that the lowest concentration of *Y. enterocolitica* that gave a response was about 10^8 cfu ml^{-1} in the peptone yeast extract-skim milk medium used. Below this bacterial level no response in conductance change was obtained.

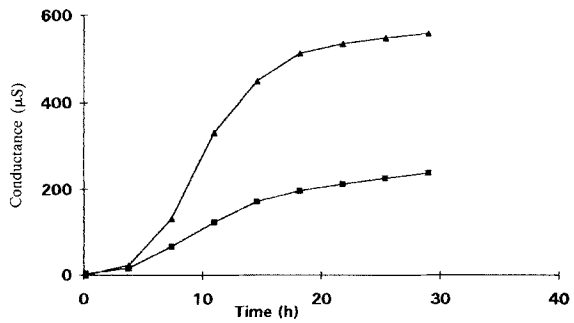


Fig. 4. Change in conductance during growth of *Y. enterocolitica* O:3 at 25 °C, pH 6.0 in SPYE-skim milk medium with 0% skim milk (■) and 5% skim milk (▲).

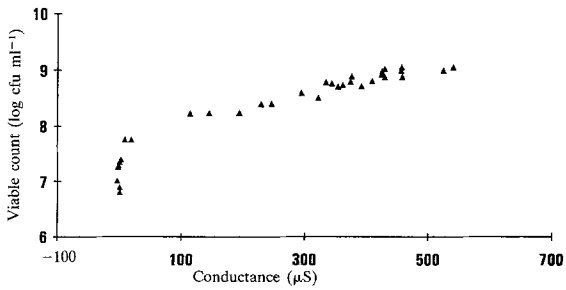


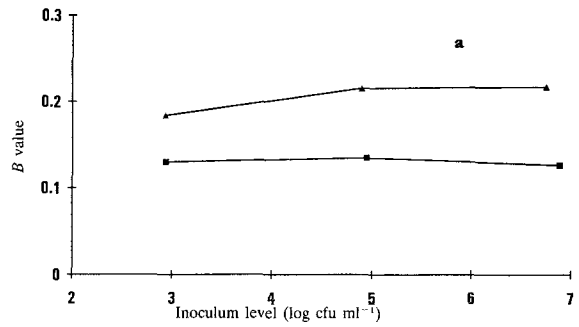
Fig. 6. Relationship between viable count and conductance dertermi-nations for *Y. enterocolitica* O:3 in SPYE-skim milk medium at pH 6.2, 18 °C.

Inoculum level

The effect of the inoculum level on the Gompertz curve parameters *A,B,C* and *M* was evaluated. The inoculum levels of *Y. enterocolitica* used were 3, 5 and 7 log cfu ml^{-1} and varying growth conditions (pH 5.4 and pH 6.2) were applied. The *B* parameter was not affected by the inoculum level (Fig. 7a), nor was the *C* parameter affected by the inoculum level. The *M* parameter (time at maximum growth rate) was, as expected, affected since the lower the inoculum level the longer the time until the threshold of the instrument is reached (Fig. 7b).

Gaseous atmosphere in the Malthus cell

It is essential to control, or at least to have knowledge of, the gaseous atmosphere prevailing during incubation. For salmonella, conductance changes under microaerophilic conditions were larger, compared to aerobic conditions [9]. The gaseous atmosphere prevailing in the test cell will depend on several factors, for example the medium volume used and the metabolic activity of the bacterial cells. A bacteria consuming oxygen and producing carbon dioxide may alter the gaseous composition of the cell during incubation. The levels of CO_2 and O_2 in a Malthus cell were monitored during incubation of *Y. enterocolitica* at pH 6.2 and 18 °C (Fig. 8). The CO_2 level increased to about 7% and a corresponding decrease in O_2 was obtained.



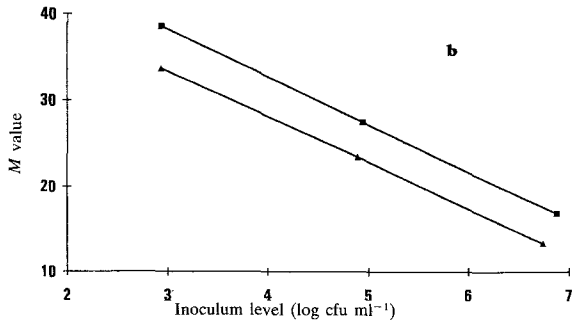


Fig. 7. Effect of inoculum level of *Y. enterocolitica* on (a) *B* parameter and (b) *M* parameter. The *B* and *M* parameters were derived using the Gompertz equation. A SPYE-skim milk medium with (■) pH 5.4, 0% L-lactate; (▲) pH 6.2, 0% L-lactate was used.

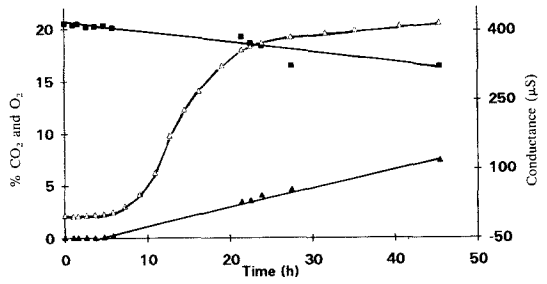


Fig. 8. Conductance change (△), CO_2 concentration (▲) and O_2 concentration (■) during incubation of *Y. enterocolitica* O:3 in SPYE-skim milk medium, pH 6.2 and 18 °C.

Polynomial model

A polynomial model based on conductance data describing the effect of pH value (5.4, 5.9, 6.2, 6.5), temperature (7, 12, 15, 18, 23 °C) and L-lactate concentration (0, 0.4, 0.8, 1.2%) on the *A, B, C* and *M* Gompertz parameters was developed for *Y. enterocolitica*. Growth occurred for all combinations. A high correlation was found between the *B* values predicted with the polynome and the *B* values observed in the Gompertz curve fittings. In Fig. 9, predicted *B* values are plotted versus the observed *B* values. Similarly high correlations were obtained for the *C* and *M* values. Thus, *B, C* and *M* values may be predicted using the models.

Predicting the growth of Yersinia *in meat*

The polynomial model describing the effect of pH, temperature and L-lactate level on the growth for *Y. enterocolitica* was tested on pork inoculated with *Y. enterocolitica*. The analyzed values of L-lactate together with the pH values and the applied temperature, were used in the polynomial models for Gompertz parameters *B* and *C* based on conductance measurements, and the conductance growth rates *BC/e* were predicted. Four values were calculated from the four experiments. Growth rates predicted from the conductance-based polynomial models are plotted versus the observed growth rates of *Y. enterocolitica* in minced pork

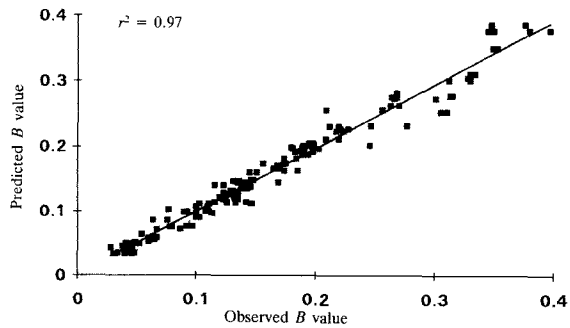


Fig. 9. Relationship between *B* values predicted with a conductance based polynomial model, and *B* values observed in the Gompetz curve fitting.

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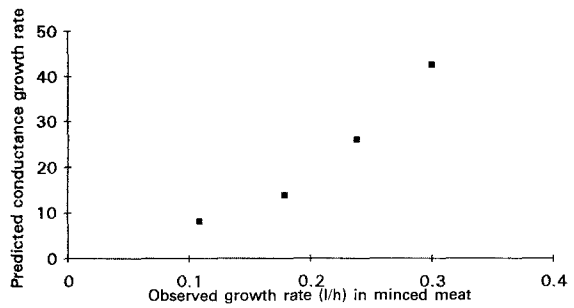

Fig. 10. Growth rates calculated from the conductance based polynomial models are plotted versus the growth rate of *Y. enterocolitica* in minced pork based on viable count determinations.

based on the viable count determinations in Fig. 10. The correspondence obtained indicates that the derived polynomial models based on conductance measurements reflect the growth of *Y. enterocolitica* and may be used for estimating the growth rate. However, further validation of the conductance models is necessary.

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