Food Engineering Progress.
Vol. 2, No. 3, pp. 171~177 (1998)

*Saccharomyces cerevisiae*의 생육 Kinetics에 미치는 전기적 처리의 영향

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Effects of Electrical Treatment on Growth Kinetics of *Saccharomyces cerevisiae*

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Abstract

The effect of electrical treatment on the growth of *Saccharomyces cerevisiae* in a simple fermentor with ohmic heating system was studied. We applied alternating electric fields of 15 V and 60 Hz to the fermentor during the entire fermentation period. The lag period decreased slightly by the use of electricity, but was not significantly different ($p>0.05$) from the heating method. The maximum growth rate constant, generation time, and maximum growth of *S. cerevisiae* were not affected significantly by the electrical treatment. The production of alcohol increased slightly by the use of ohmic heating. Results show that measurement of electric current when ohmic heating is done at a constant voltage maybe used in monitoring such fermentations. In conclusion, growth patterns of the yeast were not affected significantly under ohmic heating, but alcohol production was stimulated. Electrical treatment is potentially useful in certain applications related to fermented foods.

Key words: *Saccharomyces cerevisiae*, electrical treatment, growth kinetics, ohmic heating

Introduction

In recent years, the world's food industry has shown renewed considerable interest in technologies utilizing electrical energy directly into processing. Research in this area provides the processor with the opportunity to produce new, high quality, shelf-stable products with alternative sterilization techniques. Today, applications of electric fields have been widened to morphology, genetics, biochemistry, physiology, biotechnology etc.. Interaction of microorganisms with the electric fields has intrigued food microbiologists for many years. Most previous studies on this interaction dealt with microbial inactivation. Palaniappan *et al.* (1992) reported no significant lethal effect by electricity at field strengths commonly associated with ohmic heating. However, sublethal electrical pretreatment of food has sometimes reduced the subsequent thermal inactivation requirement. At high-field strengths associated with electroporation techniques and high-pulsed electrical fields (Palaniappan *et al.*, 1990: Castro *et al.*, 1993: Pothakamury *et al.*, 1995: Zhang *et al.*, 1994), microorganisms are inactivated, probably due to disruption of the cell membrane.

Information about the interaction of sublethal electric fields with growth and metabolic activity of microorganisms is currently limited. *In vitro* studies of low frequency electric and electromagnetic fields have revealed various effects, depending on electrical parameters on the one hand and physiological state of cells on the other (Berg, 1993). Varieties of effects have been reported: both inhibition and stimulation of cell growth (Rowley, 1972: Shimada and Shimahara, 1977: Fiedler *et al.*, 1995: Cho *et al.*, 1996), increased productivity (Hongo and Iwahara, 1979: Grosse *et al.*, 1988: Kerns *et al.*, 1993), changes in respiration processes (Berg, 1987: Berg, 1993: Beschkov and Peeva, 1994) etc..

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Impedance measurement methods (Firstenberg-Eden and Eden, 1984: Ci *et al.*, 1997) have been used for automated monitoring of microbial growth and metabolism. The changes of impedance are associated with changes in medium composition following growth of microorganism. However, sometimes contradictory results have been reported in these area, because the targets and experimental conditions are not always identical. Sublethal ohmic heating is potentially useful for doing electrical treatments in fermentation of viscous and particulate media. Homogeneous heating of such media may be achieved easily when ohmic rather than conventional heating is applied. Accordingly, the aim of this research is to study the growth kinetics of a selected yeast under ohmic, compared with conventional, heating methods. Additionally, possible alternation of the metabolic activities of this yeast by electrical treatments using the sublethal ohmic heating system was considered.

Materials and Methods

Organism

Saccharomyces cerevisiae was obtained from a commercial strain of Fleischmann's Yeast (ATCC 7754, Burns Philp Food Inc., USA) and was used throughout this study. The culture was maintained as a frozen stock at -20°C in Tryptose broth (Difco, Detroit, MI) containing 10% glycerol (Sigma Chemical Co., St. Louis, MO). Before use, the culture was inoculated into Tryptose broth at 1% level and incubated at 30°C for 24 h in a shaker. This was followed by a second transfer under the same conditions.

Ohmically Heated Fermentor

An experimental fermentor for doing electrical treatment with ohmic heating system is shown in Fig. 1 and described in detail in Cho *et al.* (1996). The time, pH, temperature, OD_{610}, voltage and current data were collected by a data logger (ISAAC 91A, Cyborg Co., Newton, MA) linked to a microcomputer. For fermentation under conventional heating, a similar fermentor assembly was used except the device for supplying electricity.

Ohmic and Conventional Fermentation

Fermentation was carried out in a fermentor equipped with conventional or ohmic heating devices. Ohmic heating was achieved by passing alternating current of 60 Hz such that a constant voltage of 15 V

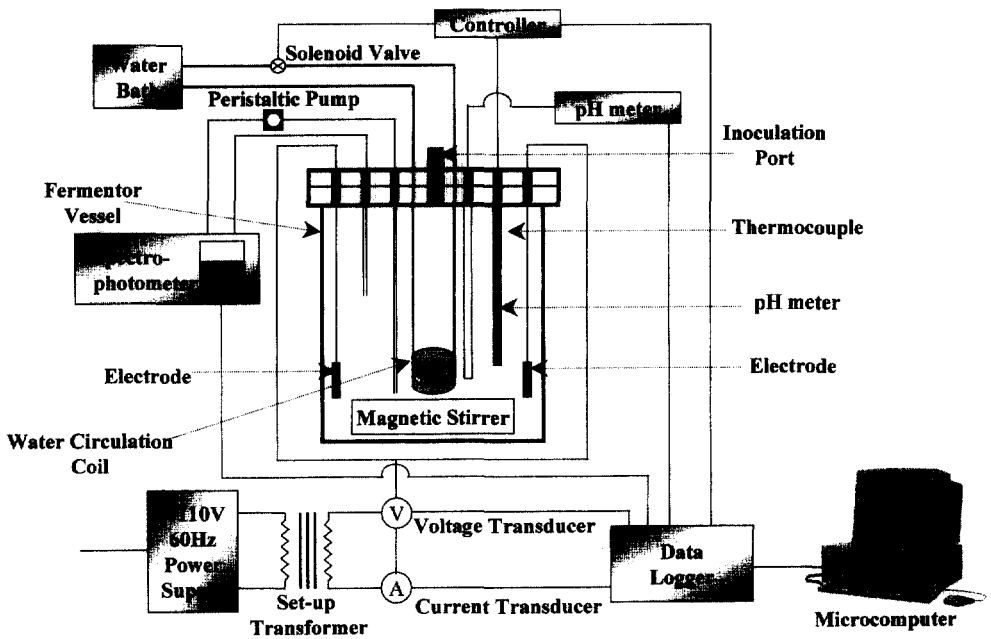

Fig. 1. Schematic diagram of the experimental setup.

was supplied. A constant temperature was maintained during fermentation under ohmic heating by intermittently circulating cooling water through the water circulation coil. The flow of cooling water was adjusted by a controller (MIC 2000 series, The Partlow Co., New Hartford, NY). The temperature of the cooling water was 10~15°C. For conventional heating, the fermentor was maintained at a constant temperature by continuously circulating controlled-temperature water through the water circulation coil. Three temperatures (20, 25, and 30°C) were tested in these experiments. The fluctuations around the set temperature were ±0.3 and ±0.5°C for conventional and ohmic heating, respectively. The average room temperature during these fermentations was 22°C.

The fermentation vessel was filled with 2.0 L of Tryptose broth and autoclaved at 121°C for 20 min. The medium in the fermentation vessel was agitated for 30 min prior to inoculation using a magnetic stirrer. The culture of *S. cerevisiae,* which was prepared as indicated earlier, was inoculated at 0.01% (v/v) of the medium volume. Data were collected during fermentation at 30 minutes interval by a microcomputer. Changes in metabolic activity were determined in samples from the fermentations at 25°C; these samples were collected manually into sterile culture tubes at 2~5 hours interval.

Assay Methods

Samples of fermented medium were centrifuged at 12,000 g for 20 min. Cell-free supernatant was filtered through a 0.45-μm-pore-size filter (Gelman Sciences, Ann Arbor, MI). Glucose and ethanol in culture filtrate were analyzed with high performance liquid chromatography (Ringbom *et al.*, 1996: Cho *et al.*, 1996). The HPLC system (Waters Associates, Inc., Milford, MA) consisted of an autosampler (Waters, WISP 712), an ion exclusion column (HPX-87X, Bio-Rad, Richmond, CA), a programmable multiwavelength detector (Waters, model 490), a differential refractormeter (Waters, model 410), and a 386-class microcomputer loaded with a data acquisition software (Waters, Maxima 820 workstation). A 10-μL culture filtrate, or dilutions thereof, was injected into the HPLC and eluted with 0.005 M H_2SO_4 which was pumped at a flow rate of 0.6 mL/min.

Data Analysis

Fermentations were repeated three times for each treatment, and the averages of growth parameters were reported. Data on metabolic changes at 25°C (i.e., glucose consumption and ethanol production) were obtained from two trials. The cell growth was monitored by measuring the optical density of cultures at 610 nm (OD_{610}). The cell counts [$\log_{10}$ of the colony forming units (CFU)/mL] was calculated from OD_{610} readings using a calibration curve, which was obtained as follows: Cells of *S. cerevisiae* were collected from 250 mL of fermentation broth by centrifugation and resuspended in 10 mL of distilled water. A series of dilution were then made from the concentrated cell suspension and OD_{610} was measured. Counts of *S. cerevisiae* in this series of cell suspensions were determined by plating appropriate dilutions onto Plate count agar (Difco, Detroit, MI) plates. The plates were incubated at 30°C for 48 h and colonies were counted. A linear relationship between $\log_{10}$ (OD_{610}) and $\log_{10}$ (CFU/mL) was obtained and used to estimate the cell counts in subsequent experiments. Growth data were fitted by the followings:

(1) Monod's model (Han and Floros, 1998):

$$\ln (Y_2/Y_1)=\mu (X_2-X_1) \quad (1)$$

where X is the time (in hours), Y is the number of cells (in CFU/mL), and μ is the growth rate constant (in $hours^{-1}$). The growth rate constant was calculated by the average value of Eq. (1) of three replications at the logarithmic growth stage, to give the linear slope. The maximum growth rate constant (μ_{max}) was determined by the maximum value of the growth rate constant.

(2) Gompertz model (Zwietering *et al.*, 1992: Cho *et al.*, 1996):

$$Y_g=A+C \exp (-\exp(-B(X-M))) \quad (2)$$

where Y_g is $\log_{10}$ (CFU/mL), X is the time (in hours), and A, B, C, and M are the model parameters. The "Nonlin" module of the Systat statistical program (Systat, Inc., Evanston, IL) was used to estimate the parameters of Eq. (2). Growth parameters were calculated as follows (Cho *et al.*, 1996): Lag period

(in hours) equals M-(1/B), minimum generation time (in hours) equals $(\log_{10}2)e/(BxC)$, and maximum growth (in $\log_{10}$(CFU/mL)) equals A+C.

Data on growth parameters were analyzed statistically using the General Linear Model of the SAS statistical program (SAS Institute Inc., Cary, NC). The multiway analysis of variance was performed using trial, method of heating, and temperature of the fermentation as treatment factors. Methods* Temperature interaction was also included in the statistical model. When treatment factors were significant, Tukey's studentized range test was used for multiple comparison of means.

Results

Electrical Treatment for Fermentation

The growth pattern of *S. cerevisiae* and a condition of electrical treatment are shown in Fig. 2. The electrical current passing in the fermented medium at 25°C, under a constant voltage of 15 V and a frequency of 60 Hz, decreased with the progress of growth. Most of the changes in the current occurred at the early exponential phase. Some of the variability in the measured current may be caused by the noise from the electrical source, the high ionic strength of the medium, and lack of accuracy and sensitivity in measuring devices at the level of current used in this study (1.43~1.53 A). However, it may be concluded from Fig. 2 that current measurements follow approximately the changes in the growth of *S. cerevisiae* during fermentation.

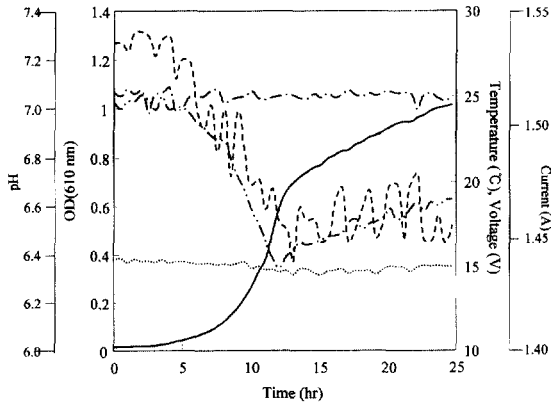

Fig. 2. Changes in voltage (···), temperature (−·), current (---), pH (−···) and growth (—) during fermentation under ohmic heating.

Growth Characteristics

Differences in growth characteristics of *S. cerevisiae* were observed when Tryptose broth was fermented under ohmic and conventional heating (Tables 1 and 2, and Fig. 3). Statistical analysis of data is shown in Table 1, and the average values of growth parameters and the statistical comparison of means at 0.05 probability (p) level are shown in Table 2. The maximum growth rate constant was not affected significantly ($p>0.05$) by the method of heating and the interaction between the method of heating and temperature of fermentation. However, an increase in temperature of fermentation increased the maximum growth rate constant significantly ($p<0.01$, Tables 1 and 2).

The lag period decreased slightly by the use of ohmic, rather than conventional, heating. But it was not affected significantly ($p>0.05$) by the method of heating and the interaction between these two factors. The generation time for *S. cerevisiae* was not significantly different ($p>0.05$) by the heating method. An increase in temperature of fermentation,

Table 1. Analysis of variance for growth parameters as affected by method of heating and fermentation temperature

Source	Degree of freedom	Mean squares	Probability (p)
Maximum growth rate constant			
Trail	2	0.000	0.497
Method	1	0.000	0.527
Temperature	2	0.047	0.000
Methods*Temperature	2	0.000	0.661
Lag period			
Trail	2	0.107	0.468
Method	1	0.515	0.075
Temperature	2	10.922	0.000
Methods*Temperature	2	0.090	0.525
Generation time			
Trail	2	0.033	0.369
Method	1	0.037	0.294
Temperature	2	2.129	0.000
Methods*Temperature	2	0.025	0.463
Maximum growth			
Trail	2	0.194	0.065
Method	1	0.032	0.459
Temperature	2	0.052	0.412
Methods*Temperature	2	0.055	0.394

Table 2. Growth parameters for *S. cerevisiae* during fermentations under ohmic and conventional heating

Heating method	Fermentation temperature (°C)	Growth parameter[a]			
		μ_{max} (h^{-1})	Lag period (h)	Minimum generation time (h)	Maximum growth (log_{10} CFU/mL)
Treatment					
Conventional	20	0.155^{+}	5.126^{+}	1.947^{+}	7.377^{+}
Ohmic	20	0.154^{+}	4.510^{+}	1.951^{+}	7.400^{+}
Conventional	25	0.278^{+}	3.579^{+}	1.085^{+}	7.249^{+}
Ohmic	25	0.270^{+}	3.424^{+}	1.114^{+}	7.180^{+}
Conventional	30	0.327^{+}	2.242^{+}	0.191^{+}	7.094^{+}
Ohmic	30	0.327^{+}	1.198^{++}	0.131^{++}	7.392^{+}
Main factors					
a. Method					
Conventional		0.253^{+}	3.649^{+}	1.238^{+}	7.240^{+}
Ohmic		0.251^{+}	3.310^{+}	1.328^{+}	7.324^{+}
b. Temperature					
	20	0.155^{+}	4.818^{+}	1.949^{+}	7.388^{+}
	25	0.274^{++}	3.501^{++}	1.099^{++}	7.215^{+}
	30	0.327^{+++}	2.120^{+++}	0.801^{+++}	7.243^{+}

[a]: Means within each data column with the same superscripts are not significantly different at α=0.05.

however, decreased the lag period significantly (p< 0.01, Tables 1 and 2).

Interestingly, the maximum growth was not affected significantly (p>0.05) by the method of heating, temperature of fermentation, and the interaction between these two factors (Tables 1 and 2).

Ethanol Production

Trpytose broth was fermented at 25°C for 25 h under ohmic and conventional heating, and changes in glucose and ethanol concentrations were monitored (Fig. 3). The pattern of glucose consumption in ohmic heating was not different from that in conventional heating. However, the ethanol production increased slightly by the use of ohmic, rather than conventional, heating.

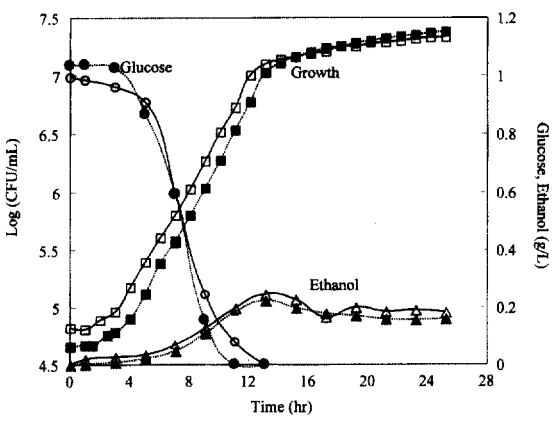

Fig. 3. Changes in growth, glucose, and ethanol during fermentation of Tryptose broth by. *S. cerevisiae* at 25°C under ohmic (—) and conventional (···) heating.

Discussion

In recent years, the low frequency (LF) or d.c. electrostimulation of cell proliferation reveals positive and negative electric effects for many animal and bacterial cells (Berg, 1987 and 1993: Cho *et al.*, 1996), whereas for yeasts only few results have been published until now: *C. albicans*, d.c. (below 57 V/cm; 20 mV/cell), inhibition of germ tube extension (cited by Fiedler *et al.*, 1995); *C. albicans*, d.c. (below 1 mA), inhibition of growth (cited by Fiedler *et al.*, 1995); *Saccharomyces cerevisiae*, a.c. (below 200 V/cm, 10 kHz), increase in cell division and orientation of budding (cited by Fiedler *et al.*, 1995). In addition to these generally positive or negative results for certain ranges of polarization of some microorganisms, specific (positive or negative) electric effects have been detected for other cells, e.g.,: *Escherichia coli* B, a.c. (0~110 mA, using a power supply source of 100 V and 50 Hz), no effect for growth lag of *E. coli* (Shimada and Shimahara, 1977); *Brevibacterium flavum*, d.c. (200~300 μA/cm^2, 1.5 V), a positive effect (increase, + 10%) for yield of L-glutamic acid (Hongo and Iwahara, 1979); *Acetobactor suboxydans*, d.c. (be-

tween 5 and 30 μA), a positive effect (increase, + 80%) for the specific growth rate (Beschkov and Peeva, 1994), no effect was found for human fibroblasts (HF-19), a.c. 50 Hz, 2 mT (Cridland *et al.*, 1993), but only this one magnetic flux. Comparing these results one can assume that electrical effects for proliferation can be detected at small d.c. or (induced) a.c. LF fields, but the experimental details available in the literature are insufficient.

The current study illustrates the difficulty in making generalized conclusions about the effect of electricity on yeast growth and survival. *S. cerevisiae*, used in this study, is a baker's yeast with significance to the bakery industry and human health. I used an electric current to directly heat a medium fermented by this yeast. This electrical treatment, when compared with conventional heating, decreased the lag period of *S. cerevisiae* slightly, and the generation time, maximum growth, and the maximum growth rate constant was not affected significantly ($p>0.05$). Additionally, ohmic heating and temperature of fermentation did not cause significant changes ($p>0.05$) in the maximum growth, but temperature of fermentation was affected significantly ($p<0.01$) in the generation time, the specific growth rate, and the lag period (Table 1). Therefore, we can assume that the electric current enhances the early stages of growth. It appears that the heating method caused fewer differences in growth patterns and metabolic activities of *S. cerevisiae*.

Glucose consumption and production of ethanol were used as indicators of the metabolic activity during fermentation. Magnitude of glucose consumption appears to depend on the cell density in the fermented medium, but the production of ethanol increased slightly by the use of ohmic, rather than conventional, heating.

Additional investigation is needed to explain the short lag period and the trend of increasing productivity observed under ohmic heating; however, a hypothesis about this phenomenon will be discussed. As a illustration by Cho *et al.* (1996), I assume that the oscillating electric field used in this study can dislodge the polar antimicrobials (which are found at low levels in culture media) adhering to cell walls and membranes. This may have improved the absorption of nutrients and minimized the inhibitory action of fresh medium, thus shortening the lag period and increasing the production of ethanol.

This study provided evidence that heating complex fermenting media by direct application of electric current may be useful in the food industry. In conclusion, the use of electricity to maintain the temperature during fermentations altered a few growth pattern and some metabolic activities of *S. cerevisiae*. Phenomena such as decreasing the lag period and increasing production of ethanol may be utilized beneficially in the food industry. In addition, we may be able to monitor the progress of the fermentation by measuring the current passing through the fermentor when the voltage remains constant.

Acknowledgement

The author wishes to acknowledge the financial support of the Korea Research Foundation made in the program year of 1997.

References

Berg, H. 1987. Electrotransfection and electrofusion of cells and electrostimulation of their metabolism. *Stud. Biophys.*, **119(1-3)**: 17-29

Berg, H. 1993. Electrostimulation of cell metabolism by low frequency electric and electromagnetic fields. *Bioelectrochem. Bioenerg.*, **31**: 1-25

Beschkov, V. N. and L. G. Peeva. 1994. Effects of electric current passing through the fermentation broth of a strain Acetobacter suboxydans. *Bioelectrochem. Bioenerg.*, **34**: 185-188

Castro, A. J., Barbosa-Canova and B. G. Swanson. 1993. Microbial inactivation of foods by pulsed electric fields. *J. Food Proc. Preserv.*, **17**: 47-73

Cho, H.-Y., A. E. Yousef and S. K. Sastry. 1996. Growth kinetics of *Lactobacillus acidophilis* under ohmic heating. *Biotechnol. Bioeng.*, **49**: 334-340

Ci, Y.-X., J. Feng, Z.-W. Jiang and D.-Z. Luo. 1997. The voltametric behavior of *Saccharomyces cerevisiae*. *Bioelectrochem. Bioenerg.*, **43**: 293-296

Cridland, N., R. Saunders and T. Cragg. 1993. *Electricity and magnetism in biology and medicine*. M. Blank (ed.), San Francisco Press, San Francisco, CA.

Fiedler, U., U. Grobner and H. Berg. 1995. Electrostimulation of yeast proliferation. *Bioelectrochem. Bioenerg.*, **38**: 423-425

Firstenberg-Eden, R. and G. Eden. 1984. *Impedance microbiology*. Wiley, New York

Grosse, H.-H., E. Bauer and H. Berg. 1988. Electrostimulation during fermentation. *Bioelectrochem. Bioenerg.*, **20**: 279-285

Han, J. J. and J. D. Floros. 1998. Modeling the growth

inhibition kinetics of baker's yeast by potassium sorbate using statistical approaches. *J. Food Sci.*, **63**(1): 12-14

Hongo, M. and M. Iwahara. 1979. Application of electro-energizing method to L-glutamic acid fermentation. *Agric. Biol. Chem.*, **43**(10): 2075-2081

Kerns, G., E. Bauer and H. Berg. Electrostimulation of cellulase fermentation by pulsatile electromagnetically induced currents. *Bioelectrochem. Bioenerg.*, **32**: 89-94

Palaniappan, S., S. K. Sastry, and E. R. Richter. 1990. Effects of electricity in microorganisms: A review. *J. Food Proc. Preserv.*, **14**: 393-414

Palaniappan, S., S. K. Sastry, and E. R. Richter. 1992. Effects of electroconductive heat treatment and electrical pretreatment on thermal death kinetics of selected micro-organisms. *Biotechnol. Bioeng.*, **39**: 225-232

Pothakamury, U. R., A. Monsalve-Gonzalez, G. V. Barbosa-Canova and B. G. Swanson. 1995. Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric field technology. *Food Res. Int.*, **28**(2): 167-171

Ringbom, K., A. Rothberg and B. Saxen. 1996. Model-based automation of baker's yeast production. *J. Biotechnol.*, **51**: 73-82

Rowley, B. A. 1972. Electrical current effects on *E. coli* growth rates. *Proc. Soc. Exptl. Biol. Med.*, **139**(3): 929-934

Shimada, K. and K. Shimahara. 1977. Effect of alternating current on growth lag in *Escherichia coli* B. *J. Gen. Appl. Microbiol.*, **23**: 127-136

Zhang, Q., A. Monsalve-Gonzalez, G. V. Barbosa-Canova and B. G. Swanson. 1994. Inactivation of *E. coli* and *S. cerevisiae* by pulsed electric fields under controlled temperature conditions. *Trans. ASAE.*, **37**(2): 581-587

Zwietering, M. H., F. M. Rombounts and K. van't Riet. 1992. Comparison of definitions of the lag phase and the exponential phase in bacterial growth. *J. Appl. Bacteriol.*, **72**: 139-145