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BIPHASIC THERMAL DEATH TIME CURVES AT HIGH AND ULTRAHIGH TEMPERATURES OF BACILLUS SUBTILIS HU 1 SPORES

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SUMMARY

Spores of Bacillus subtilis HU1 suspended in whole milk and /or distilled water subjected to high and ultrahigh temperatures ranging from 85 to 140°C. Resulting survivor and thermal death time (TDT) curves were shown to be non-linear for all temperatures investigated; convex survivor curves at most heating temperatures were observed. TDT curves observed in all studies were concave and biphasic. The z-value using milk as the menstruum were 57°C for heating temperatures of 121-140°C, and 20°C, for heating temperatures of 110-121°C. Distilled water as the menstruum yielded z-values of 58°C and 16°C for heating temperatures of 120-140°C and 110-121°C respectively.

INTRODUCTION

Many research workers have obtained non-linear heat survivor curves. Concave and/or convex curves, for example, were reported (Licciardello and Nickerson, 1963; Shull *et al.*, 1963; Fox and Eder, 1969; Al-Khayat, 1974; Al-Horainy, 1979). Thermal death time (TDT) curves were also reported with two slopes showing both low and high

z-values (Edwards *et al.*, 1965; Al-Horainy, 1979). The TDT curves have been constructed by either taking the D-values from the linear portion of the convex/concave (El-Bisi and Ordal, 1956 a,b, Fox and Eder, 1969), or applying the equation of Stumbe (1948). Biphasic TDT curves have been discussed in relation to consecutive reaction (Al-Khayat, 1974; Hermann *et al.*, 1978).

The research describes in this paper, the method of how determine the D- and z-values for Bacillue subtilis HU1 at high and ultrahigh temperatures in different heating menstrua.

MATERIALS AND METHODS

Organisms and spore cultures of B. subtilis HU1 were obtained from the Microbiology Collection (Bereich allgemeine Mikrobiologie of Humboldt University, Berlin). This strain grew well on nutrient agar (Oxoid) fortified with minerals, which was used by Pelcher *et al* . (1963) but without potassium dihydrogen phosphate. The final prepared medium was designated as fortified nutrient agar (FNA). This medium yields spore-free sporangia and reduces the requirements of lysozyme treatments (Busta and Ordal, 1964). Nutrient broth (Oxoid) was used as an activation medium for the inoculum before adding to the sporulation media. All incubations were carried out at 37°C.

Production of Endospores

Active inocula were prepared by three successive 24 hour subcultures using nutrient broth. The last subculture was dispensed on to the surface of Roux flasks containing 200 ml FNA, using 2.3 ml portions. The flasks were incubated at 37°C for one week during which time period sporogensis was monitored by phase microscopy. The

resultant spore crop was harvested using cold sterile distilled water and subsequently pooled. The spore suspension was shaken with glass beads (2mm) for 24 hours at 5°C in order to reduce clumping, and then passed through a sterile sintered glass filter to remove any remaining spore clumps. The spore suspension was washed with cold sterile distilled water and centrifuged at 3000 rpm. This process was repeated seven times; the final centrifugation was carried out at 8000 rpm (Stewart and Halvorson, 1953; Long and Williams, 1956; Busta and Ordal, 1964; Nath and Clegg, 1969). The final washed spore pellet was resuspended in a final volume of 50 ml cold sterile distilled water. This stock suspension was maintained at 4°C until used.

Spore heat treatment and enumeration

Spore suspensions were prepared from the stock using both sterile distilled water and sterile homogenized whole milk. The heating menstruum (3 ml) contained a final concentration of 10^5-10^6 spores/ml (non-heat activated counts). All trials were performed in 12-14 mm diameter screw-capped vials using a thermostatically-controlled glycerin bath. At predetermined intervals of time, vials were removed from the bath and rapidly cooled in ice water.

Viable cell counts were determined using purred plate technique (NA, Oxoid) serial dilutions. Plates were incubated for 24 hours at 37°C. All results represent the average of three treatments.

RESULTS

Survival curves determined at 85-140°C for water are shown in Fig. 1,2 and 3, and for milk in Fig. 4 and 5. All of these curves appear to deviate from the

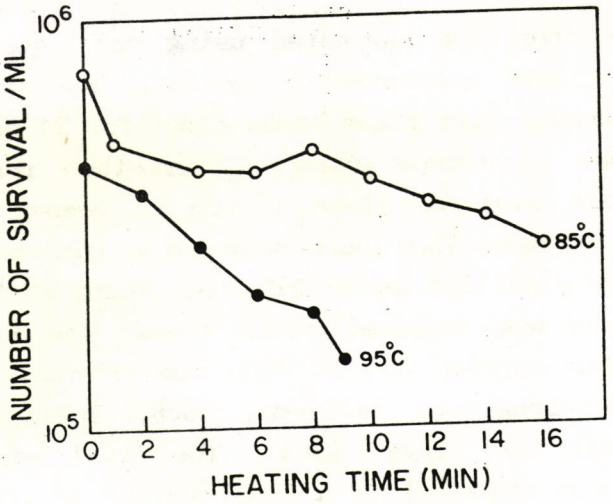

Fig.1. Survival curves for spores heated in water at 85•C and 95•C.

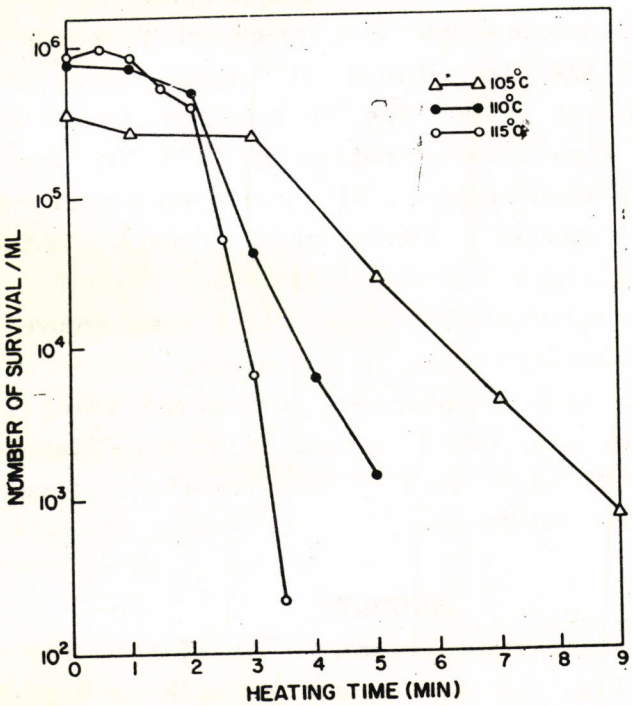

Fig.2. Survival curves for spores heated in water at 105•C, 110•C and 115•C.

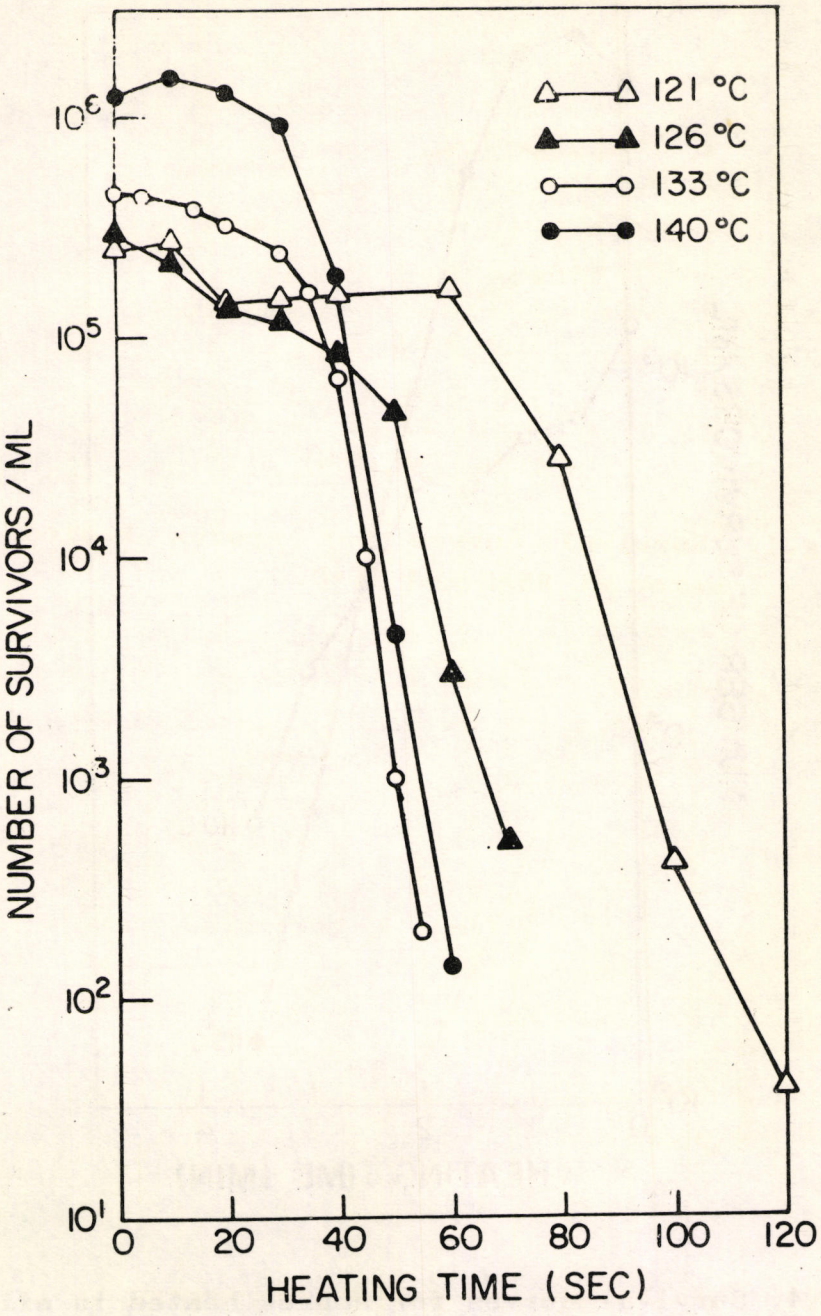

Fig.3. Survival curves for sopres heated in water at 121°C, 133°C and 140°C.

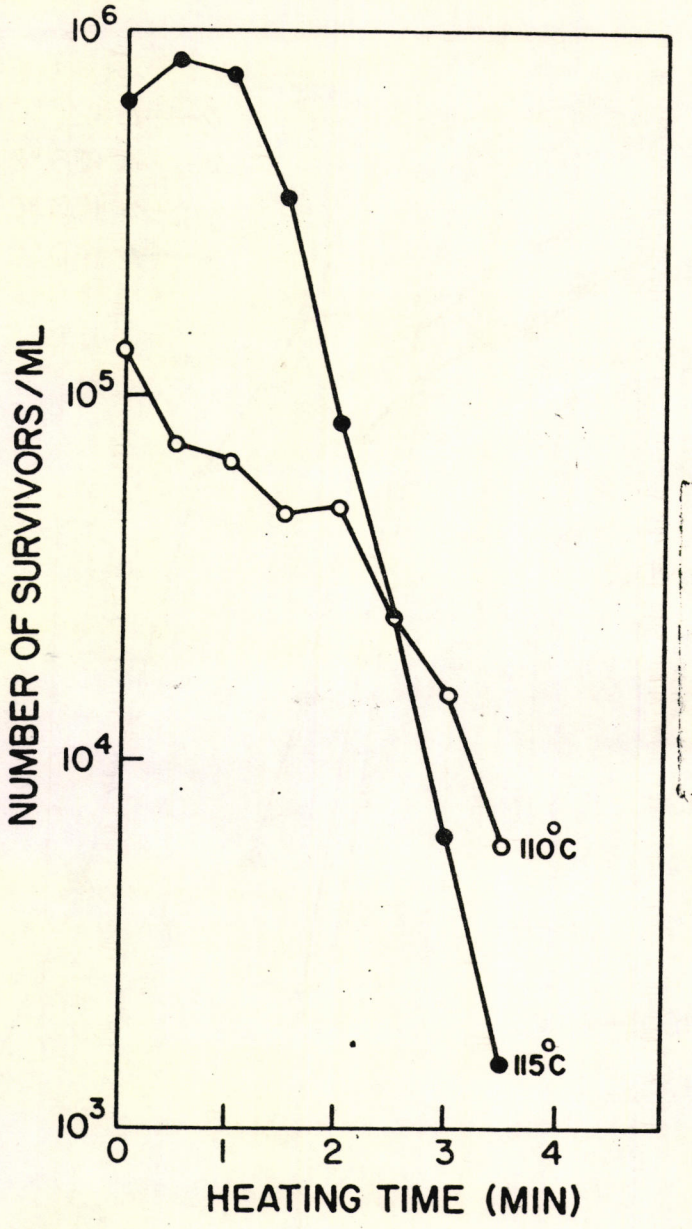

Fig.4. Survival curves for sopres heated in milk at 110•C and 115•C.

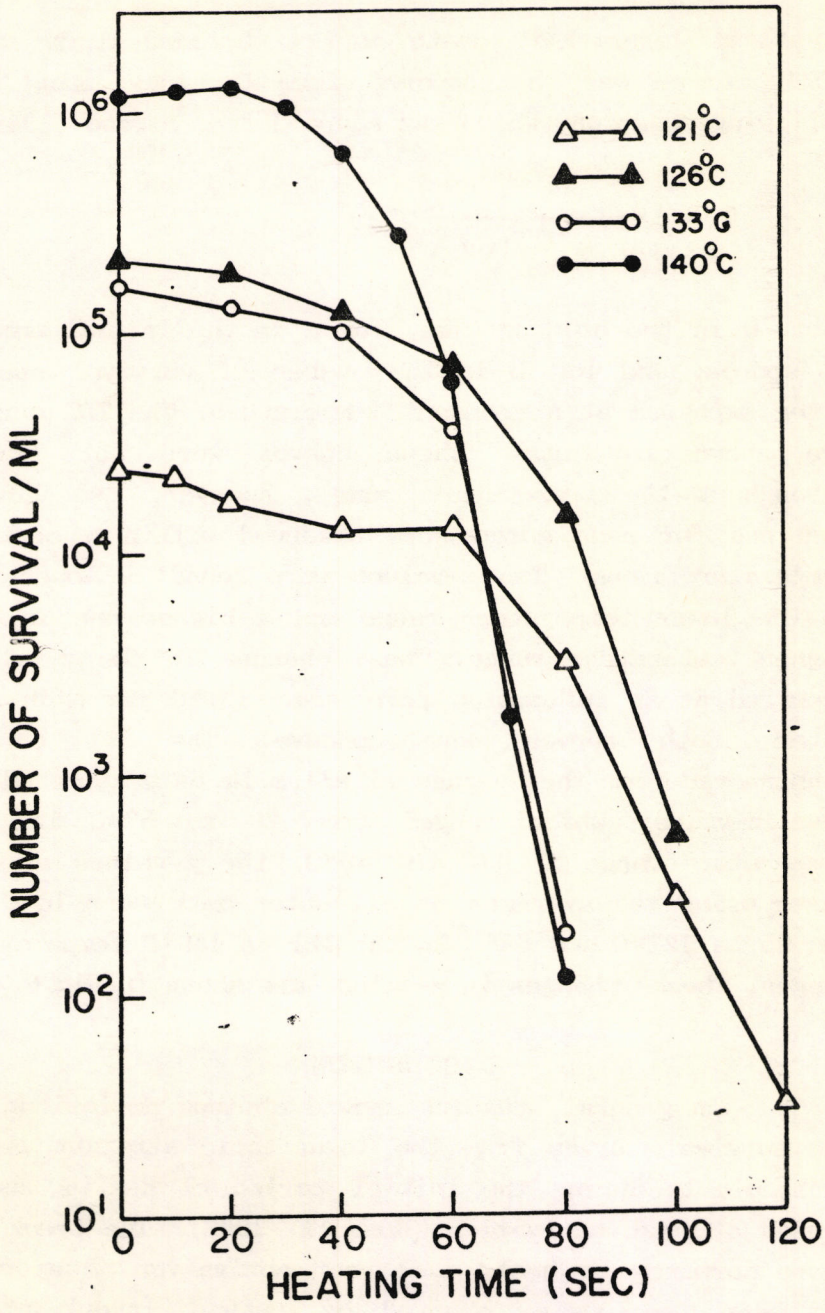

Fig.5. Survival curves for sopres heated in milk at 121°C, 126°C 133°C and 140°C.

classical logarithmic death curves. Thermal death time (TDT) curves were constructed from D-values using the following equation, which was adapted from Stumbo (1948):

$$D = \frac{U}{\log a - \log b}$$

where U is the holding time, log a is the initial number of spores, and log b is the number of survival spores after exposure at a constant temperature. The TDT curves are shown in Fig.6. These curves were not linear throughout the temperature range; however, two linear portions for each curve were observed within a certain temperature range. Two z-values were found: a lower one in the lower temperature range and a higher one in the higher temperature range. These changes in the z-values occurred at an inflection point near 121°C for milk and water. Both curves were concave. The TDT curves constructed from the average of all milk data appeared to have z-values which ranged from 20 to 57°C in the temperature range of 110 to 140°C. the z-values of the curve using the averages of all water data were 16°C in the 85 to 121°C and 58°C in the 121 to 140°C temperature ranges. Theses changes in z-values are shown in Fig.6.

DISCUSSION

In general, results showed obvious deviations of the survival curves from the logarithmic straight line, particularly during the initial period of heating as a reason of heat activation (Russell, 1982). The break of spore dormancy by heat, i.e. heat activation of spores, had been previously reported by several investigators (Curran and Evans, 1945, 1946, 1947; Fox and Eder, 1969, Al-Khayat, 1974). In previous thermal inactivation work,

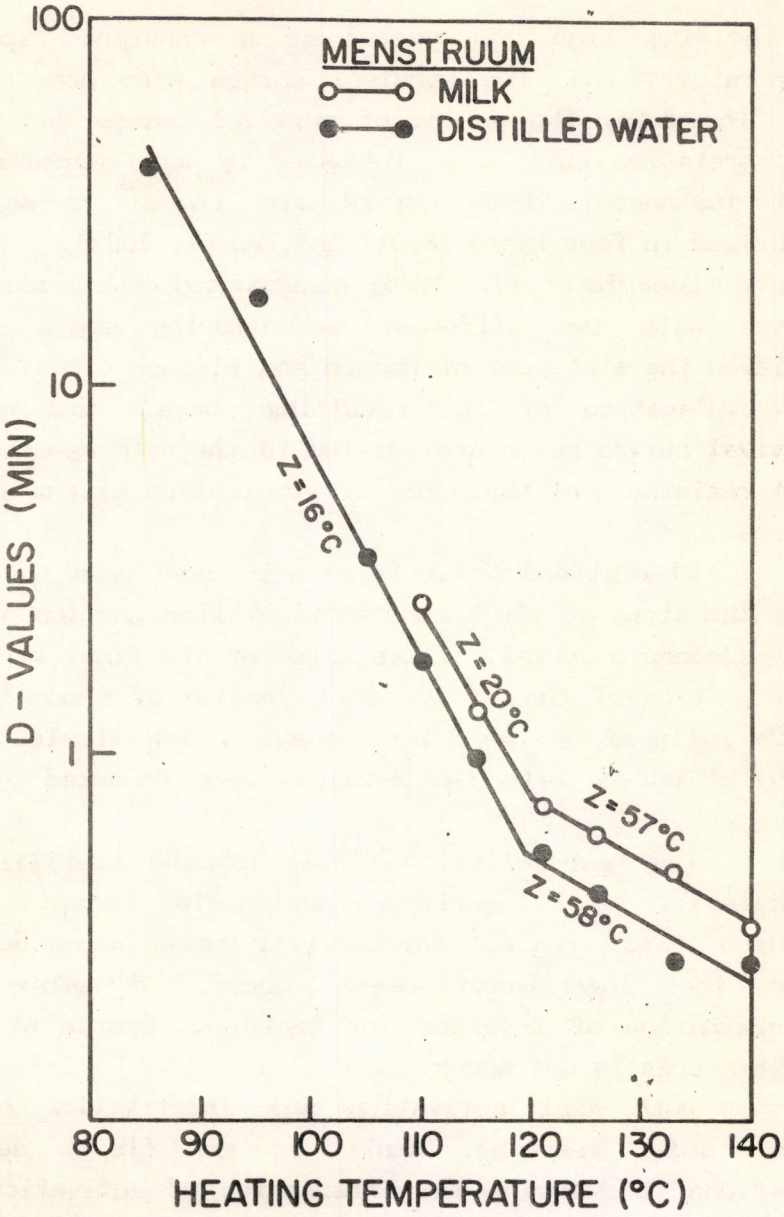

Fig.6. Thermal death time curves for sopres heated in water and milk.

the holding time was varied at a constant exposure temperature, and time survival curves were constructed from the data. The nature of survival curves and their interpretation have been discussed by many workers. In most instances, these curves are linear or may be described in four types (Shull and Ernest, 1962).

Edwards *et al.* (1965) constructed convex survival curves with two different subculturing media. They believed the statement of Hansen and Riemann (1963) about the explanation of the resulting concave and convex survival curves which are related to the heterogeneity in heat resistance of the spore of germination system within spores.

El-Bisi and Ordal (1956 a,b) have used the data from the slope of the second straight line portion of the convex/concave curves, or the slope of the final straight line portion of the curves which consist of a non-linear curve followed by the linear segment. Two straight-line survival curves with two D-values were reported by Fox and Eder (1969).

The mathematical analysis of the sterilization process for ideal logarithmic destruction is well known (Stumbo, 1973), but not for survival curves which deviate from the logarithmic death curve. Therefore the determination of D-values for bacterial spores at high temperatures is not accurate.

Both heat activation and inactivation obeyed first order reaction. Shull *et al.* (1963) derived equations to describe the interaction of activation and inactivation of the thermal treatment of spores. The activation and inactivation rate constants were calculated from the survivor curve without relating them to the temperature dependence. Herrmann *et al.* (1976,

1978) explained the kinetics of heat activation and thermal death of bacterial spores as consecutive, i.e.,

$$N_o \xrightarrow{k_1} N^+ \xrightarrow{k_2} N^-$$

where N_o = the number of initial spores, N^+ = the number of activated spores at a constant temperature, N^- = the number of killed spores, and k_1 and k_2 are the activation rate constant and the inactivation rate constant respectively. All the deviations from the non-logarithmic death were discussed and calculated through the difference between the assumed number of initial already-activated spores and the number of nonactivated spores and also the relation between k_1 and k_2 (Al-Khayat, 1974; Herrmann *et al* . 1978). The determination of z-values for the ultrahigh temperatures can not obtained through the extrapolation of one slope of TDT curves. There is a point on which the TDT curve will be deviated to another slope, if the bacterial spores heated for a side range and over 100°C temperatures (Edwards *et al.*, 1965; Al-Khayat, 1974; Srimani *et al.*, 1980). If the D-values are determined from the nonlogarithmic death, then the z-values become smaller than that determined from the equation of Stumbo (194) (Fig.6). This equation gives a two slope curve of "biphasic slope" of TDT curve, when the D-values are determined at elevated temperatures as used in ultrahigh temperature sterilization processes.

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منحنيات وقت القتل الحراري (TDT) في درجات الحرارة العالية والعالية جدا لسبورات بكتريا BACILLUS SUBTILIS HUL

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الخلاصـــة

تم تعريض سبورات البكتريا Bacillus Subtilis Hul المعلقة في الحليب الكامل او الماء المقطر الى درجات حرارة عالية جدا تتراوح مابين 85 الى 140 درجة مئوي. منحنيات القضاء على السبورات الناتجة غير مستقيمة لكل درجات الحرارة المستخدمة لوحظت المنحنيات المحدبة لمعظم درجات الحرارة. منحنيات الـ TDT كانت في كل الدراسة مقعرة وعلى مرحلتين. قيمة الـ Z في الحليب كانت 57 درجة مئوي لدرجات الحرارة 140-121 درجة مئوي، و 20 درجة مئوي لدرجات الحرارة 110 الى 121 درجة مئوي. اما في الماء المقطر فكانت قيمة الـ Z 58 درجة مئوي و 16 درجة مئوي لدرجات الحرارة 120 الى 140 درجة مئوي وكذلك 110 الى 121 درجة مئوي على التوالي.