The Effect of Temperature on the Growth of *Acinetobacter Calcoaceticus*

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Abstract

Acinetobacter calcoaceticus. a bacterium which plays an important role in waste water treatment processes, was cultured in a temperature gradient incubator over a temperature range of ca. 16–40°C to determine the temperature-growth relationship of this micro-organism. The optimum temperature was in the region of 29–35°C, and a maximum specific growth rate of 1,28 h^{-1} was reached. Arrhenius plots of growth against temperature were drawn, and a distinct inflection point was discerned at 23,5°C, which indicated an abrupt increase in activation energy below this temperature. Activation energies, Q_{10} values and Arrhenius equations were calculated. A temperature range of 29–35°C would seem to be desirable for waste treatment processes employing *A. calcoaceticus*.

Introduction

Eutrophication is a potential major water quality problem in South Africa (Toerien, 1975). Reducing the nitrogen and phosphorus inputs from point sources such as sewage treatment plants will in the future rely on modified activated sludge processes (Bolitho, 1976). Bacteria of the genus *Acinetobacter* play an important role in the biological removal of phosphorus in such activated sludge systems (Fuhs and Chen, 1975). Previous work (Du Preez, 1975) has indicated that *Acinetobacter* was also effective in removing C_2–C_5 fatty acids from a fatty acid-rich effluent produced by the Sasol coal to oil conversion. *Acinetobacter* is therefore an important bacterium in waste water treatment processes, and the optimal utilization of this micro-organism in such processes depends on a basic knowledge of its growth requirements and kinetics.

The purpose of this study was to determine the temperature – growth relationship of *Acinetobacter calcoaceticus*.

Materials and Methods

Micro-organism and culture media

The bacterium *Acinetobacter calcoaceticus* was isolated from silt from the H.F. Verwoerd dam by enrichment culture technique using a medium containing acetate as the major carbon source (Du Preez, 1975).

Two culture media were used: (a) an acetate medium containing, per litre distilled water, $CH_3COO{\cdot}Na{\cdot}3H_2O$, 9,2 g; $(NH_4)_2SO_4$, 5,0 g; sodium dihydrogen citrate, 0,3 g; K_2HPO_4, 5,0 g; KH_2PO_4, 5,0 g; $MgSO_4{\cdot}7H_2O$, 0,5 g; $CaCl_2{\cdot}2H_2O$, 0,05 g; $FeSO_4{\cdot}7H_2O$, 0,02 g; $ZnSO_4{\cdot}7H_2O$, 7,0 mg; $MnSO_4{\cdot}4H_2O$, 5,0 mg; $Na_2MoO_4{\cdot}2H_2O$, 2,0 mg; $CuSO_4{\cdot}5H_2O$, 0,25 mg; $CoSO_4{\cdot}7H_2O$, 1,0 mg; H_3BO_3, 0,3 mg. Adjusted to pH 6,7. (b) Tryptone soy broth (TSB) (Lab M, London) containing, in g per litre, tryptone, 17,0; soy peptone, 3,0; NaCl, 5,0; K_2HPO_4, 2,5; dextrose, 2,5. To this 2,5 g KH_2PO_4 was added for extra buffering capacity, and the pH adjusted to 7,0.

The media were autoclaved at 121°C for 15 min. All chemicals used were of reagent grade.

Apparatus

The experiments were conducted by growing the bacterium in a temperature gradient incubator (Scientific Industries Inc., New York) consisting of an aluminium bar, one end of which is heated and the other cooled to produce a stable temperature gradient. Two sets of thirty horizontal sample wells are situated in parallel at equidistant points along the bar. The bacterial cultures were contained in L-shaped test tubes (30 ml, 17 mm diameter) of optically selected glass, which fit into the sample

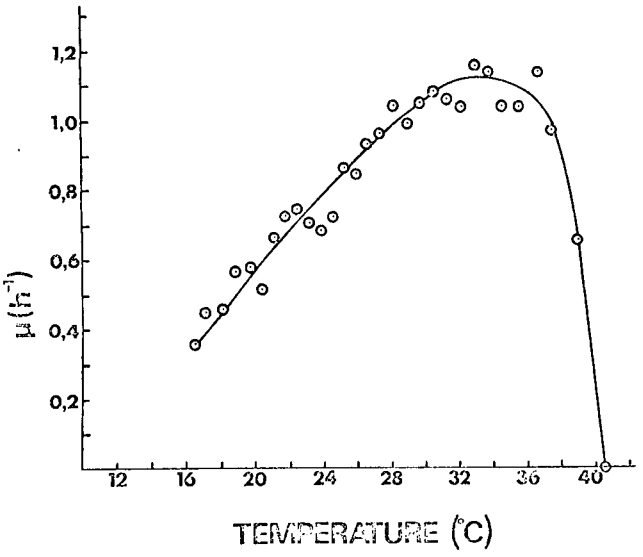

Figure 1
The effect of incubation temperature on the specific growth rate of Acinetobacter calcoaceticus *in an acetate medium.*

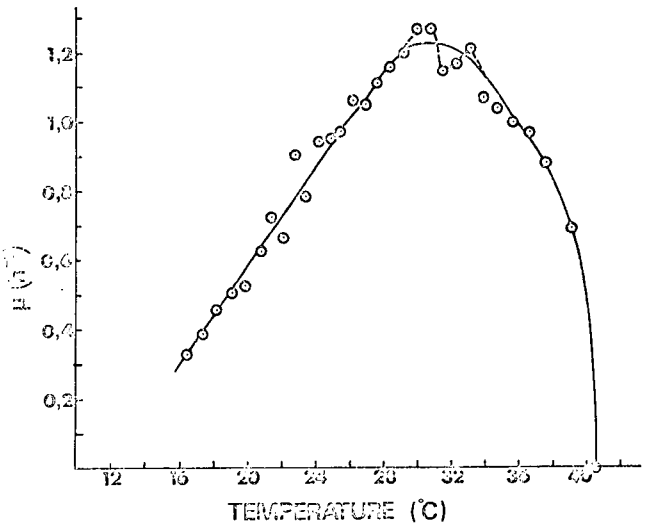

Figure 2
The effect of incubation temperature on the specific growth rate of Acinetobacter calcoaceticus *in tryptone soy broth.*

wells. The tubes were capped with either cotton plugs or with loose-fitting metal caps. Aeration and agitation were provided by a built-in shaker rocking the bar through a 30° arc at 40 oscillations per min. In every experiment the culture tubes, each containing 10 ml of the appropriate sterile medium, were placed in the polythermostat overnight to allow temperature equilibration prior to inoculation. Each culture tube was inoculated with 1,0 ml from a 16 h shake flask culture of *A. calcoaceticus* in TSB incubated at 30°C.

Growth measurements

Growth was followed by measuring the turbidity of the culture in a Klett-Summerson colorimeter (Klett Mfg. Co., New York) at ca. 640 nm (red filter). The culture tubes were removed sequentially without stopping the shaker, thus disrupting growth for the minimum period of time. Exponential growth usually occurred between 30–200 Klett units. Since a linear relationship was found to exist between the dry cell mass and Klett units up to at least 250 Klett units, it was possible to calculate the growth rate directly from the Klett readings. Growth rate was expressed as the specific growth rate, μ, where

$$\mu = (\ln X_2 - \ln X_1) / (t_2 - t_1)$$

and X_1 and X_2 were the cell concentrations at time t_1 and t_2 respectively. The specific growth rates were calculated by plotting the logarithm of the absorbance (Klett units) against time and substituting the values from the exponential portion of the growth curve into the above equation. Experiments were performed in triplicate with the acetate medium and in duplicate with TSB.

Calculation of activation energy

The Arrhenius equation, $\mu = Ae^{-E/RT}$ or $\log_{10}\mu = \log_{10}A - (E/2{,}303R)^1/T$ where μ is the specific growth rate, A an entropy constant, E the activation energy, R the gas constant, and T the absolute temperature, was used to calculate the activation energy or temperature characteristic (Pirt, 1975). For this purpose Arrhenius plots of log μ against the reciprocal of the absolute temperature were drawn, and the slope of the line equalled E/2,303R.

Results and Discussion

Effect of temperature on growth rate

When the culture tubes were capped with cotton plugs variable results were obtained, and at the higher growth rates (μ>0,8 h^{-1}) growth was linear rather than exponential, which was possibly due to oxygen limitation as suggested by Brock (1974). Satisfactory results were obtained when the cotton plugs were replaced with loose-fitting metal caps. This observation emphasized the importance of adequate aeration in studies of this nature.

The variation in temperature over a 10 h period, the duration of each experiment reported here, was 0,7°C in the terminal tubes next to the heated and cooled ends of the aluminium bar. The maximum variation in the more central tubes did not exceed 0,2°C. This variation was considered to be insignificant in the evaluation of the results.

The effect of temperature on the growth rate in the acetate medium is shown in Figure 1, where each point represents the mean of triplicate experiments. The temperature optimum was in the region of 30–36°C which was in agreement with previous results (Abott *et al.* 1973) for the same species grown in an ethanol-containing medium. The pH of the acetate medium changed from an initial value of 6,7 to 8,6 during growth, and this may have contributed to the scattering of the points in Figure 1. The experiment was repeated with TSB, where only a slight change of pH (from 7,0 to 7,2) occurred during growth. These results are shown in Figure 2, where each point represents the mean of duplicate experiments. The scattering of the points was markedly reduced. The general shapes of the growth curves depicted in Figures 1 and 2 differed somewhat. In the acetate medium *A. calcoaceticus* reached a maximal

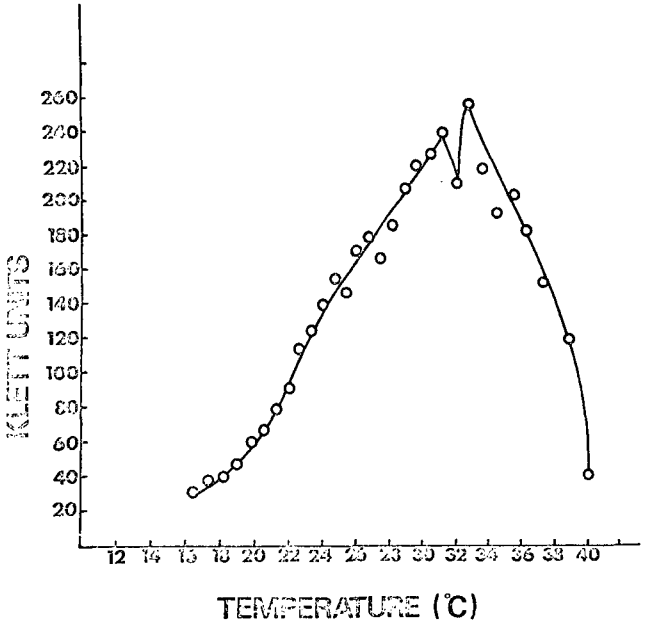

Figure 3
The effect of temperature on the growth of Acinetobacter calcoaceticus *in tryptone soy broth as measured by turbidity after 3 h incubation.*

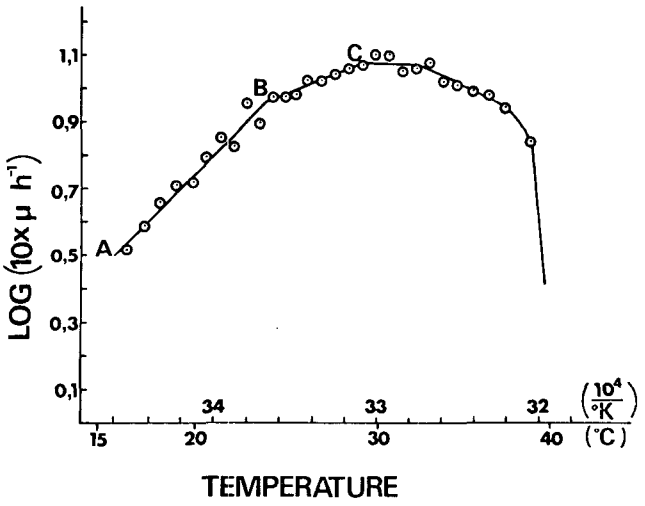

Figure 4
An Arrhenius plot of the relationship between growth rate and temperature for Acinetobacter calcoaceticus *in tryptone soy broth, calculated from the data of Figure 2.*

growth rate in the range of 30–36°C and thereafter the growth rate declined precipitously with no growth at 40,6°C. However, in the TSB the maximal growth rate was reached at 29 to 33°C; from 33 to ca. 37°C the growth rate declined rapidly, and above 37°C the decline was precipitous with no growth at 40,6°C. A slightly higher maximum growth rate was reached in TSB (1,28 h^{-1}) than in the acetate medium (1,17 h^{-1}), possibly due to the different compositions of the two media.

Although not specifically determined, extrapolation of the curves in Figures 1 and 2 suggested that the minimum growth temperature of the bacterium would be ca. 11–12°C.

Temperature-related growth anomaly

Various researchers have suggested that abrupt changes in the properties of water and aqueous solutions occur near 15, 30, 45 and 60°C, ± 2°C (Oppenheimer and Drost-Hansen, 1959; Davey *et al.* 1966), and it has been postulated that these anomalies in the structure of water influence the behaviour or activity of biological systems, e.g. the growth of micro-organisms. In Figure 3 is shown the turbidity after 3 h incubation in TSB against temperature. A slight dip in the curve, indicative of slower growth, could be observed in the region of 32°C. A duplicate experiment gave similar results. In Figure 2 it is possible to discern a slight dip in the curve at 31–32°C (broken line), also indicating a suppression of growth rate. Although these observations apparently agree with the results of Davey *et al.*. (1966) and Oppenheimer and Drost-Hansen (1959) for other bacteria, they are of much smaller magnitude, suggesting that some bacteria, e.g. *A. calcoaceticus*, are less sensitive towards changes in the properties of water with temperature than others.

Arrhenius plots

Arrhenius plots of growth in the TSB and acetate medium are presented in Figures 4 and 5. In TSB the growth rate increased with increasing temperature up to point C, and a distinct inflection was discerned at 23,5°C (point B), indicating an abrupt increase in the activation energy when the temperature fell below 23,5°C. Activation energies, as calculated by regression analysis, are presented in Table 1. Below 23,5°C in TSB the activation energy was 2,4 times as high as that in the range 23,5–29°C, indicating that *A. calcoaceticus* is better adapted for waste water treatment processes operating at temperatures above 24°C (but not exceeding ca. 35°C).

The Arrhenius plot of growth in the acetate medium (Figure 5) failed to show a distinct inflection point and an activation energy of 55,15 kjoules (13,18 kcal) was calculated for line K – M. However, if it was assumed that a similar inflection point (point L) existed as in Figure 4, activation energies could be calculated for lines K – L and L – M (broken lines) which were 73,18 and 43,35 kjoules (17,49 and 10,36 kcal) respectively. These values did not differ drastically from those obtained in TSB (Table 1). The greater variation in the results with the acetate medium could therefore have been masking the existence of such an inflection point.

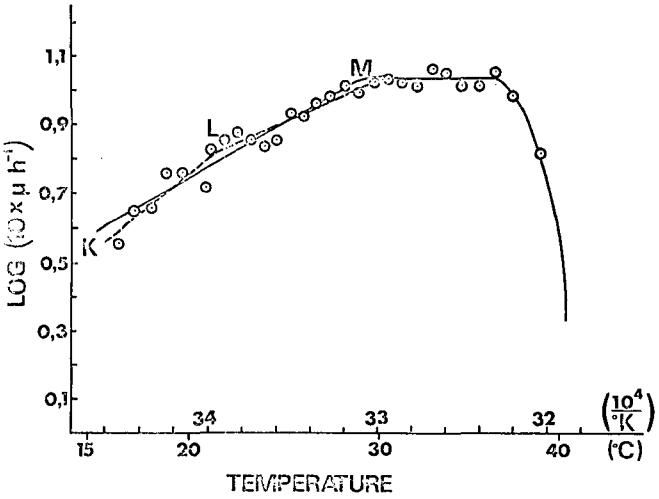

Figure 5
An Arrhenius plot of the relationship between growth rate and temperature for Acinetobacter calcoaceticus *in an acetate medium, calculated from the data of Figure 1.*

TABLE 1

ACTIVATION ENERGIES FOR *ACINETOBACTER CALCOACETICUS* CALCULATED FROM ARRHENIUS PLOTS IN FIGURES 4 AND 5.

Line	Slope	Activation energy (kjoules)	Activation energy (kcal)	Correlation coefficient (r)	Pairs of observations (n)
		Tryptone soy broth, Figure 4			
A–B	–4996	95,69	22,87	–0,97*	10
B–C	–2078	39,79	9,51	–0,97*	7
		Acetate medium Figure 5			
K–M	–2878	55,15	13,18	–0,96*	18
K–L	–3821	73,18	17,49	–0,87*	7
L–M	–2263	43,35	10,36	–0,91*	11

*Significant at $p<0,01$

TABLE 2

ARRHENIUS EQUATIONS AND Q_{10} VALUES FOR THE GROWTH OF *ACINETOBACTER CALCOACETICUS*.

Culture medium	Temperature range	Arrhenius equation	Q_{10}
TSB	Below 23,5°C	$\mu = 6166 \times 10^{13} e^{11507/T}$	3,8
	Above 23,5°C	$\mu = 9122 \times 10^{3} e^{-4786/T}$	1,7
Acetate medium	Below 22°C	$\mu = 6172 \times 10^{9} e^{-8802/T}$	2,8
	Above 22°C	$\mu = 3218 \times 10^{4} e^{-5213/T}$	1,8
	10 to 29°C	$\mu = 3679 \times 10^{6} e^{-6630/T}$	2,1

Data for *Escherichia coli* also indicated a similar inflection point occurring at 26°C (Pirt, 1975). According to Pirt (1975) such changes in the activation energy indicate differences in the rate-controlling reactions or in the metabolic regulation of cells, exemplified by the failure of β-galactosidase repression in *E. coli* below 25°C (Ng *et al.*. 1962). It is possible that the metabolism of *Acinetobacter* could also be altered by temperature changes across the inflection temperature, and this factor should be kept in mind as far as waste treatment processes are concerned.

The different Arrhenius equations and the Q_{10} values derived from them are summarized in Table 2. Biological reactions are in general considered to have Q_{10} values of ca. 2. The high Q_{10} values for growth below 23,5°C suggest that *A. calcoaceticus* is very sensitive to temperature changes in this temperature region, and processes in which the growth of this micro-organism is to be maximized (e.g. single-cell protein production) should operate above this temperature. In waste treatment processes, such as the activated sludge process where the growth rate of the sludge is selected through manipulation of the sludge age, the nett growth rate of micro-organisms is usually much less than the potential maximum growth rates. For example, the nett growth rate of an activated sludge system operating at a 10 day sludge age is only about 0,004 h^{-1}, while *A. calcoaceticus* is capable of up to 1,2 h^{-1}. Consequently the micro-organism is able to multiply rapidly in activated sludge systems (should the other environmental conditions allow such multiplication), or it can maintain itself in such systems operating at temperatures of approximately 13°C.

The growth rate is less subject to temperature influences above 23°C (but not exceeding 35°C) and the Q_{10} value is 1,7 to 1,8. If the temperature of a waste treatment process employing *A. calcoaceticus* can be controlled, a temperature of ca. 29 to 35°C seems to be desirable.

Acknowledgment

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