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Influence of Temperature on Glucose Utilization by *Pseudomonas fluorescens*[1]

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The influence of temperature on the conversion of glucose into cell material and into energy for maintenance was determined for *Pseudomonas fluorescens* by a steady-state turbidity method and by a substrate utilization method. Conversion of glucose into cell material was measured as yield; conversion of glucose into energy for maintenance was measured as specific maintenance, the minimum dilution rate in continuous culture below which a steady state is not possible. The values obtained by the two methods were nearly identical; with both, the yield and specific maintenance decreased with decreasing temperature. The specific maintenance consumption rate (milligrams of glucose taken up per milligram of cell dry weight per hour at zero growth) was also calculated by the substrate utilization method and found to decrease with decreasing temperature. However, the amount of glucose consumed per generation for maintenance increased with decreasing temperature. This increased glucose consumption for maintenance may provide a partial explanation for the decrease in yield at low temperatures. Small amounts of glucose were also converted into pigment at all temperatures tested, with the greatest amount formed at 20 C.

In another paper (Palumbo and Witter, Can. J. Microbiol., *in press*) dealing with this subject, temperature was found not to alter the pathways of glucose catabolism by the psychrophilic bacterium *Pseudomonas fluorescens*.

This study was undertaken to further the knowledge of bacterial physiology and growth as they are influenced by temperature. The objective was to determine the influence of temperature on the utilization of the substrate, glucose, by *P. fluorescens*. The aspects of glucose utilization studied included its conversion into cell material (yield) and by-products, and into energy for maintenance. The basis of this study is the frequent observation that lowering the temperature of incubation of an organism alters its metabolism. This altered metabolism may be manifested as a change in end products (4, 17, 18), an increase in pigment formation (19), or an increase in unsaturated fatty acid composition of some bacteria (10, 11, 15) and of a yeast (9).

Implicit in this investigation is the possible eludication of specific temperature-dependent or temperature-sensitive reactions. These temperature-sensitive processes could manifest themselves as major shifts in the pattern(s) of glucose utilization as the temperature of incubation is altered.

Dorn and Rahn (2) and Foter and Rahn (5) observed that the amount of lactose consumed by lactic streptococci during the doubling of one cell was constant at low and medium temperatures, but that it increased toward the optimal temperature. They concluded that bacteria utilize their food more economically at low temperatures. Ng et al. (13) found that the yield of *Escherichia coli* in batch culture (dry weight of cells formed per dry weight of limiting substrate) decreased with decreasing temperature.

Yield can readily be determined in a continuous culture system. Herbert et al. (7) provided an extensive study of continuous culture yields of *Aerobacter aerogenes* and discussed many of the factors influencing yield in continuous culture. They found that, between a minimal and a maximal dilution rate, yield was proportional to the dilution rate.

Marr et al. (12) expanded this observation and subsequently developed an equation relating steady-state turbidity (x), dilution rate (D), and specific maintenance (a). The specific maintenance represents the consumption of the carbon

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and energy source for purposes that are not a function of the growth rate (12). In a continuous culture system, a is the minimum value for the dilution rate below which a steady state is not possible. Marr et al. found that the specific maintenance in *E. coli* PS decreased dramatically when the temperature of incubation was lowered from 30 to 15 C.

The specific maintenance can also be determined in a continuous culture system by the substrate utilization method of Schulze and Lipe (16). They derived a series of equations based primarily on the rate of substrate utilized as a function of the dilution rate. They obtained a parameter called specific maintenance consumption rate (n) which is milligrams of glucose taken up per milligram of cell dry weight per hour at a dilution rate of zero.

While a is a rate function (the dilution rate below which a steady state is not possible), n represents the amount of glucose in milligrams of cell dry weight per hour needed to maintain the viability of a bacterial culture. Addition of any quantity of glucose below n will decrease the viability of the culture, and ultimately will wash out. Addition of any amount of glucose above n will allow the culture to grow to a population proportional to the amount of glucose added.

MATERIALS AND METHODS

Organism. The organism used in these studies was a psychrophilic strain of *P. fluorescens*.

Medium. *P. fluorescens* was grown in glucose-basal salts broth of the following composition: 0.6% K_2HPO_4, 0.3% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.02% $MgSO_4$, and glucose, at levels indicated later in this report. An aqueous solution of glucose was sterilized separately by autoclaving and was added to the autoclaved basal salts broth.

Specific maintenance, specific maintenance consumption rate, and yield. The specific maintenance was determined in continuous culture by the steady-state turbidity method of Marr et al. (12) and by the substrate utilization method of Schulze and Lipe (16). Yield was calculated by dividing the X_{max} (dry weight) by the limiting glucose concentration for the steady-state turbidity method. The specific maintenance consumption rate and yield were also calculated by the substrate utilization method.

The continuous culture system used in the maintenance studies was similar to that described by Akin et al. (1), except that *p*H was not controlled. To insure the adequacy of aeration for the specific maintenance cultures, the oxygen absorption rate (OAR) of the aeration portion of the continuous culture system was determined at the three experimental temperatures. The sulfite oxidation method of Ecker and Lockhart (3) was used to measure the OAR. The OAR was expressed as millimeters of oxygen per liter per hour.

Temperature. The specific maintenance, specific maintenance consumption rate, and yield were determined at 30, 20, and 8 C. The basis for choosing these temperatures was discussed elsewhere (Palumbo and Witter, Can. J. Microbiol., *in press*).

Effluent analyses. The effluent from the specific maintenance studies was monitored periodically by measuring optical density, dry weight, *p*H, and residual glucose. Optical density was measured at 340 nm in a Bausch & Lomb Spectronic 20 colorimeter against an uninoculated tube of medium. For dry weight determinations, a known volume of culture effluent was centrifuged at 5,100 × g at 2 C for 10 min; the cell pellet was then washed with distilled water, resedimented, dried at 100 C overnight (12 to 18 hr), cooled, and weighed. The supernatant fluid from the first centrifugation was used for *p*H, pigment, and residual glucose analysis. Residual glucose was determined by the Glucostat method (Worthington Biochemical Corp., Freehold N.J.). The amount of pigment (fluorescein) was determined by its absorption at 405 nm.

RESULTS

The influence of temperature on the conversion of glucose into cell material (yield) and into energy for maintenance of *P. fluorescens* was determined.

In the steady-state turbidity method of Marr et al. (12), the steady-state turbidity of a continuously growing culture was measured at various dilution rates. A steady state was established in the culture when the optical density of the effluent did not vary more than 2% over two generation times. A generation time (gt) was calculated from the equation:

$$gt = 1n\ 2/D$$

where D is the dilution rate. The steady-state turbidity (x) and the dilution rate are related by the equation:

$$(1/x) = (a/X_{max})(1/D) + (1/X_{max})$$

where a is the specific maintenance (the diversion of substrate to nongrowth functions) and X_{max} is the concentration of bacteria which can be supported if none of the substrate were used for specific maintenance, $a = 0$. A plot of the reciprocal of the steady-state turbidity versus the reciprocal of the dilution rate gave a straight line with an ordinate intercept of $1/X_{max}$ and a slope equal to a/X_{max}.

Figure 1 shows the results of a specific maintenance study of *P. fluorescens* performed at 20 C by the steady-state turbidity method. Similar graphs and calculations were obtained for the other temperatures. The specific maintenance values at 30, 20, and 8 C are 0.161, 0.110, and 0.0534 hr^{-1}, respectively.

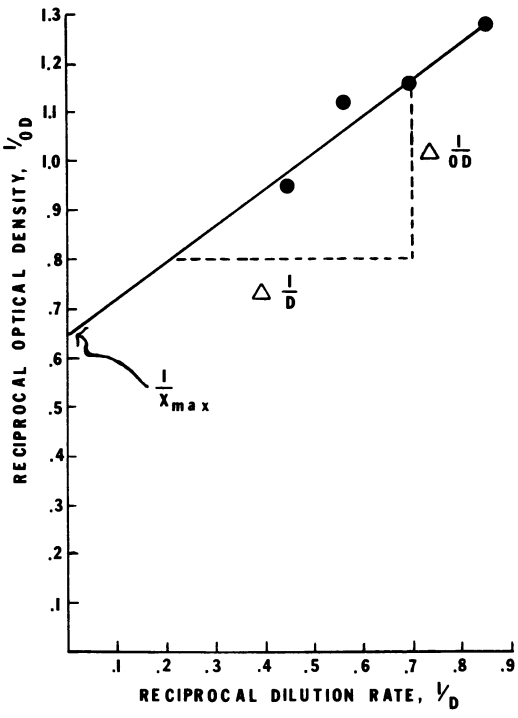

FIG. 1. *Specific maintenance of P. fluorescens at 20 C by the steady-state turbidity method. Data and calculations from the graph: $1/X_{max} = 0.650$; $X_{max} = 1.54$; slope = 0.0715 hr^{-1}; a = (slope)(X_{max}); a = 0.110 hr^{-1}. Yield (Y) is equal to X_{max} (cell dry weight) divided by the limiting glucose concentration. X_{max}, dry weight, is 0.176 mg/ml and the limiting glucose concentration is 0.333 mg/ml. Thus, Y is 0.528 mg of cell/mg of glucose.*

The steady-state turbidity method of Marr et al. (12) is dependent on the substrate (glucose) being present in the medium in limiting concentration. When glucose is limiting, a linear function with a positive slope is seen (Fig. 1), whereas a linear function with a negative slope is observed and no meaningful data can be obtained when glucose is nonlimiting. A system utilizing nonlimiting glucose had residual glucose in the effluent and gave a negative slope.

The relation of specific maintenance to temperature is given in Table 1. The specific maintenance decreased linearly with decreasing temperature. This decrease of specific maintenance with temperature is comparable to the finding of Marr et al. (12). They found a dramatic decrease in the specific maintenance of *E. coli* PS (a mesophile) when the temperature was lowered from 30 to 15 C. A less dramatic decrease in the specific maintenance was observed in our experiments with *P. fluorescens* (a psychrophile).

TABLE 1. *Influence of temperature on the maintenance requirement of P. fluorescens grown in continuous culture in basal salts broth with limiting glucose*[a]

Temp (C)	Substrate utilization method			Steady-state turbidity method		
	a	Y	n	a	Y	X_{max}
30	0.167	0.667	0.25	0.161	0.652	0.217
20	0.110	0.550	0.20	0.110	0.528	0.176
8	0.0683	0.455	0.15	0.0534	0.385	0.0385

[a] a = The specific maintenance, hr^{-1}; Y = yield constant (milligrams of cell dry weight per milligram of glucose) if no glucose were utilized for maintenance; n = specific maintenance consumption rate (milligrams of glucose taken up per milligram of cell dry weight per hour at zero growth); X_{max} = maximum population in cell dry weight (mg/ml).

From the linear relationship of log a versus 1/°K (Arrhenius plot), a temperature characteristic (μ) of 8,430 cal is calculated for the specific maintenance for *P. fluorescens*. The temperature characteristic is calculated from the slope of this linear relation by the equation:

$$\mu = (\text{slope})(2.303)(R)$$

where R is the universal gas constant. Assuming a linear relationship between temperature and specific maintenance for the data of Marr et al. (12) for *E. coli* PS, a temperature characteristic of 20,200 cal is calculated. Thus, the temperature characteristic of the response of specific maintenance to variation in temperature was considerably greater for the mesophile than for the psychrophile. This same relationship was found by Ingraham (8) for the variation in growth rate with changes in temperature; the temperature characteristic of the mesophile was greater.

The specific maintenance as well as the specific maintenance consumption rate and the yield were determined by the substrate utilization method of Schulze and Lipe (16). Figure 2 shows the results of a specific maintenance experiment performed at 20 C by this method. Similar graphs and calculations were obtained at the other temperatures.

The influence of temperature on the maintenance requirement of *P. fluorescens* grown in continuous culture as measured by both methods is given in Table 1. The specific maintenance, yield, and specific maintenance consumption rate decreased with decreasing temperature.

The OAR of the aeration portion of the continuous culture system used in the maintenance studies was determined at each of the three ex-

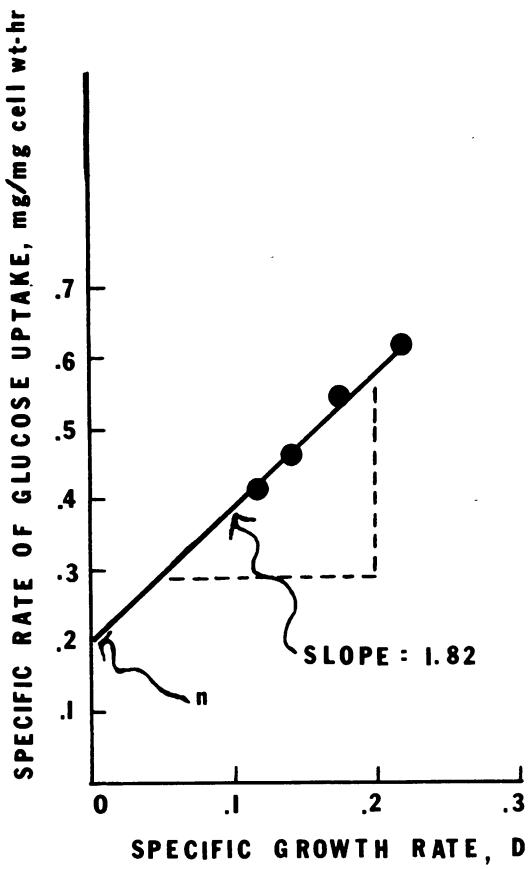

FIG. 2. *Specific maintenance of P. fluorescens at 20 C by the substrate utilization method. The plotting equation is:* $K_2 = n\ D/Y$, *where* K_2 *is the specific rate of glucose uptake in milligrams of glucose per milligram of cell dry weight per hour, n is the specific maintenance consumption rate in milligrams of glucose taken up per milligram of cell weight at zero growth* (K_2 *at* $D = 0$), *Y is the yield in milligrams of cell dry weight per milligram of limiting glucose, and D is the dilution rate. The ordinate intercept is n and the slope is equal to 1/Y. Values for* K_2 *at various dilution rates can be obtained from the equation:* $K_2 = D\ S/x$, *where S is the limiting glucose concentration and x is the cell concentration in the growth flask. The specific maintenance (a), the specific maintenance consumption rate (n), and yield (Y) are related by the equation:* $a = Y\ n$. *Data and calculation from the graph: slope = 1.82 =* $1/Y$; $Y = 0.550$ *mg of cell dry weight per mg of glucose;* $n = 0.20$ *mg per mg per hr;* $a = 0.110\ hr^{-1}$.

perimental temperatures. The OAR at 30, 20, and 8 C is 26.4, 24.8, and 22.6 mm of oxygen per liter per hr, respectively. These values for the OAR at the three temperatures were considered to provide more than adequate amounts of oxygen for the growth of *P. fluorescens*. Wright and Lockhart (20) found for *E. coli* that an OAR of 40 mm of oxygen per liter per hr would support the growth of 10^{11} cells/ml. The highest cell count in these studies was ca. 2×10^9 cells/ml. Thus, the aeration provided the cultures at each of the three temperatures is more than adequate to support the populations experienced. Difference in the OAR cannot provide the basis for the change of yield with temperature.

A partial explanation for the decrease of yield with temperature may be found in the relationship of specific maintenance consumption rate and yield. Although both decrease with decreasing temperature, the amount of glucose consumed per generation for maintenance increases with decreasing temperature. The specific maintenance consumption rate for 30, 20, and 8 C is 0.25, 0.20, and 0.15 mg of glucose per mg of cell dry weight per hr, respectively (Table 1). The corresponding maximum generation times are 2, 4, and 13 hr, respectively. Thus, the amount of glucose consumed per generation for maintenance is 0.50, 0.80, and 1.95 mg/mg of cell dry weight, respectively, for 30, 20, and 8 C. With more of the substrate required for maintenance at 8 C, less is available for conversion into cell material and, thus, the lowered yield at this temperature.

Herbert (6) and Schulze and Lipe (16) also found that maximal yields were obtained at high dilution rates. These high dilution rates represent high growth rates (shorter generation times). As indicated above, proportionally less of the substrate was involved in maintenance and more could be channeled into cell material. Temperature apparently influenced yield only by limiting the maximal growth rate at the different temperatures. Aeration was in excess of what was needed to support the populations and, therefore, cannot exert any influence on the yield.

By regulating growth rate, temperature also influenced the growth-limiting concentration of glucose. The growth-limiting glucose concentration at 30 and 20 C is 0.33 mg/ml, whereas at 8 C it is 0.1 mg/ml.

The effluent from the maintenance experiments was monitored periodically for turbidity, presence of residual glucose, and waste products to determine the course of the study (Table 2). The residual glucose in a maintenance study utilizing limiting glucose was essentially zero, or below 0.02 mg/mg, the limit of sensitivity of the Glucostat. The *p*H of the effluent from the maintenance studies at 30 and 20 C did not change, whereas that from the 8 C study dropped from *p*H 7.0 to 6.8 (Table 2). This *p*H change corresponds to 0.006 meq of acid/ml.

TABLE 2. *Influence of temperature on the limiting glucose concentration and on pH and pigment in the effluent from specific maintenance studies*

Temp	Limiting glucose concn	pH	Pigment (OD405 nm)
C	mg/ml		
30	0.33	7.0	0.009–0.018
20	0.33	7.0	0.12–0.14
8	0.10	6.8	0.009–0.014

The optimum temperature for pigment production for *P. fluorescens* grown in continuous culture is 20 C (Table 2). This observation is in agreement with the findings of Seleen and Stark (14), who found that temperatures of 20 to 30 C favored the production of the green fluorescent pigment of psychrophilic strains of *P. fluorescens*. Pigment production was observed at all three temperatures, even though the glucose (the energy and carbon source) was present in growth-limiting concentrations. Pigment production under these conditions of growth-limiting glucose may indicate that pigment has some metabolic function rather than being formed as a waste product or as a result of a shunt mechanism.

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