Influence of Temperature on Substrate and Energy Conversion in *Pseudomonas fluorescens*

RANDALL H. MENNERT AND T. O. M. NAKAYAMA

Department of Food Science, University of Georgia, Athens, Georgia 30601

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The influence of temperature on yield, maintenance rate, growth rate, and conversion of calories to biomass was studied with *Pseudomonas fluorescens* grown in a chemostat. Maintenance and growth rate are influenced linearly with temperature. Both rates increased with increasing temperature and gave linear Arrhenius plots over a limited range. Cells harvested during the steady-state at each temperature were burned in a microcalorimeter. The number of kilocalories per gram (dry weight) of organism was not influenced significantly by the temperature during growth, indicating that the conversion of substrate calories into biomass is apparently regulated in the range of temperature studied.

Over 30 years ago, Foter and Rahn (14) observed that temperature affected the amount of substrate utilized per cell. This was one of the earliest papers correlating total growth and temperature. In more recent experience, yield (weight of cells produced per weight of substrate utilized) has been shown to decrease with decreasing temperature (25, 26, 28).

Temperature also affects the maintenance requirement of microorganisms. Specific maintenance rate has been shown to give linear Arrhenius-van't Hoff plots over a limited temperature range (22). There is evidence that at some temperatures respiration becomes uncoupled from growth (6, 15, 31). Even the chemical composition of the microbial cell is thought to be influenced by incubation temperature (4, 33).

Thus temperature has considerable influence on all phases of microbial physiology. A profound change in the physiology of the cell should be reflected in the caloric balance, i.e., the caloric input and the caloric output. This study was undertaken to examine the influence of temperature on growth rate, maintenance rate, yield, ash content, and the heat of combustion of bacterial cells. On the basis of the data obtained, the question of an energy budget is raised.

**MATERIALS AND METHODS**

**Organism and substrate.** The organism *Pseudomonas fluorescens* B1351 was obtained from Robert Weaver of the National Center for Disease Control, Atlanta, Ga. Stock cultures were maintained on Brain Heart Infusion slants stored at 4°C (L. J. Guarratia, personal communication). Cultures were transferred at least monthly.

Experimental growth was carried out in the basal salts/glucose media employed by Condon and Ingraham, with two modifications: the nitrogen source was increased by 10% and nitrilotriacetic acid was deleted. The medium consisted of KH₂PO₄, 6.8 g; Na₂HPO₄, 7.1 g; (NH₄)₂SO₄, 1.1 g; MgSO₄·7H₂O, 615 mg; CaCl₂·2H₂O, 79 mg; (NH₄)₁₆Mo₇O₂₄·4H₂O, 0.2 mg; Hutner's metals (7), 1.0 ml; glucose, 1.0 g; and distilled water to 1.0 liter. The medium is limiting only in carbon source. No pH control was attempted. The original pH was 6.65 and did not change, except for very densely packed cultures in which the pH decreased to 6.62 to 6.60.

**Growth apparatus.** The growth chamber, filters, reservoir, air pump, and collection tube are essentially the same as described by Eiler and Webb (13). The apparatus was purchased from the Kontes Glassware Co., Vineland, N.J. (catalogue no. K88104). The chamber was modified to have a water jacket and larger capillary (3 mm) on the outflow. Capacity of the growth chamber was 286 ml.

Flow rates were maintained by keeping a constant head between the level of medium in the reservoir and a solenoid valve. Flow rates were adjusted by changing the time between pulses and the length of the pulse to the solenoid. Rate (time between successive pulses) and duration (time length of pulse) were programmed on an electric timer. Both solenoid and timer were made by the electronic shop at the University of Georgia. The error in flow was less than ±2%; over the entire range of experimental flow rates. A schematic diagram of the chemostat is shown in Fig. 1.

The air pump pushes a 9-inch (22.9 cm) head (Hg) and gasses the system at the rate of 178 liters/hr. With these measurements, an estimation of power input was made with the method of Ecker and Lockhart.

**References**

1. Presented to the American Chemical Society at the national meeting in Chicago, Ill., on 17 September 1970. Journal series no. 958, College of Agriculture, Experiment Station, Athens, Ga. 30601.
Steady-state cells were harvested by centrifuging at 20,000 \( \times g \) for 20 min at 0 to \(-2^\circ C\) on a Sorvall RCB-2 refrigerated centrifuge. The cells were washed twice with cold (0 to 4 \( C \)) distilled water. The twice-washed cells were freeze-dried under clean but not sterile conditions. Calorimeter pellets were made of the freeze-dried cells and placed in tared planchetts with the platinum pans necessary for burning. The pellets were then dried to constant weight at 65 \( C \) and 260 mm of Hg. Benzoic acid standards were dried at 75 \( C \) and 760 mm. The bomb was standardized at every cell burning in at least quintuplet. Our reported calorimeter values for the cells represents no less than 10 burnings in each case on the Phillipson Micro Bomb Calorimeter (Gentry and Weigert Instruments, Aiken, S.C.).

Cell ashings were performed by transferring dry cell powder to tared crucibles and drying to constant weight at 65 \( C \) and 260 mm. The cells were ashed to constant weight at 800 \( C \) and appeared snowy white. All final results are given in Table 2.

**RESULTS**

**Growth-maintenance rate data.** At dilution rates well below the maximum (ca. 0.5 or less), the cultures were unstable, and maintaining a steady-state was very difficult. It was possible to operate as a turbidostat at low dilution rates but not as a chemostat with a constant dilution rate. For this reason, the maintenance rate of 35 \( C \) could not be accurately estimated. Table 1 shows the experimental relationship between cell concentration and dilution rate.

Maintenance rates were found by plotting inverse optical density versus inverse dilution rate according to the equation of Marr et al. (22): 

\[
1 / x = (a / x_{\text{max}}) / D + 1 / x_{\text{max}}
\]

where \( x \) is the bacterial concentration, \( D \) the dilution rate in hr\(^{-1} \), \( a \) is the maintenance rate in hr\(^{-1} \), and \( x_{\text{max}} \) is the bacterial concentration that would be sup-

**Table 1. Relationship between steady-state dilution rate and bacterial concentration**

| Temp (C) | \( D \) (hr\(^{-1} \)) | \( A_{\infty} \) |
|----------|------------------------|--------------|
| 20       | 0.149                  | 0.236        |
|          | 0.185                  | 0.252        |
|          | 0.205                  | 0.260        |
|          | 0.228                  | 0.270        |
|          | 0.250                  | 0.277        |
| 25       | 0.250                  | 0.422        |
|          | 0.282                  | 0.443        |
|          | 0.333                  | 0.465        |
|          | 0.404                  | 0.490        |
|          | 0.420                  | 0.495        |
| 30       | 0.414                  | 0.361        |
|          | 0.545                  | 0.397        |
|          | 0.630                  | 0.418        |

**Fig. 1. Schematic diagram of the chemostat.**

(12). The power input was found to be 0.2 w/liter. Mixing at high flow rates was found to be virtually instantaneous so that chemical homogeneity was accomplished and there was no measurable heat lag with incoming medium. A Tamson water bath and Neslab PBC-4 bath cooler were used to control temperature.

The oxygen absorption rate (OAR) was determined at 30 \( C \) by the sulfite oxidation method (9, 11). The OAR was 91 mmole of \( O_2 \) per liter per hr. To support a population of \( 10^9 \) cells/ml, an OAR of 40 mmole of \( O_2 \) per liter per hr was found to be in excess of the requirement (35). Pirt (30) found that in continuous culture there is little difference between OAR values of 24, 85, and 225. Hence, 91 mmole per liter per hr was deemed sufficient.

Analytical methods and operation. Dry weight estimations were made at each temperature employed. The analyses were carried out by taking washed cells, diluting them to arbitrary optical densities in distilled water, and monitoring them at 660 nm. Portions (25 ml) of each sample were transferred, in triplicate, to tared 100-ml beakers and evaporated to constant weight at 65 \( C \) and 260 mm of Hg. A constant flow of air was used during the drying process to prevent fogging and splattering. Water blanks were included.

Cells were adapted twice to basal salts/glucose medium before inoculation of the chemostat. The standard inocula consisted of 2 ml of a logarithmic culture with an absorbancy at 660 nm (\( A_{\infty} \)) between 0.7 and 1.0. For growth rate and maintenance rate determinations, the chemostat was allowed to reach approximately one-third of its maximum possible density before feed was started. Maximum specific growth rates were found by the "wash-out" method described in detail by a number of investigators (17, 27).

Specific maintenance rates were determined by the steady-state turbidity method (22). A steady-state was defined as \( \pm 2\% \) cell concentration over two generations at a given dilution rate. Generation times were calculated from \( D \) (or \( k \)) = \( \ln 2 / g \), where \( D \) is the dilution rate equal to the growth rate \( k \), in hr\(^{-1} \), and \( g \) is the generation time in hr.

Yields were ascertained by stopping the flow, allowing the cells to come to 90 to 95\% of their maximum density, and then starting the feed at the maximum dilution rate. After removing the cells, residual substrate analyses were performed by the phenol-sulfuric acid method (10).
conversion was estimated. The expression of Baas-Becking and Parks (1) was changed from 100 × (Eo/Ea) to 100 × (∆H0/∆H0)Y, where E0 is the heat of combustion of the cells formed, equal to ∆H0, and Ea is the free energy produced by metabolism of the substrate. The term E0 was replaced with ∆H0, the heat of combustion of the substrate. Y is the yield factor. All data are presented in Table 2.

**DISCUSSION**

From the data obtained for temperature characteristics of growth and maintenance, it appears that maintenance processes are only slightly more temperature-dependent than growth. The factors influencing maintenance have been reviewed by several researchers (15, 21, 31). The high un is a result of processes other than diffusion (transport) being involved in maintenance. A temperature characteristic of maintenance of 20 kcal/mole has been found for *E. coli* and a similar conclusion was drawn (22).

The trend of lower yield with lower temperature is observed in *P. fluorescens*. Yields given here, however, are higher than have been reported for directly calculated yields of various *Pseudomonas* species on glucose in minimal medium. Reported yields range around 40% for aerobically grown batch cultures (18, 20, 24, 29). It is interesting to note that, although yield diminishes with decreasing temperature, so does the specific maintenance rate, and in fact the ratio a/kmax changed very little. Hence, an increased requirement for maintenance is not the cause of smaller yields.

It has been reported that temperature affects the chemical composition of cells (4, 25, 32, 33). Gross changes in composition should give different heats of combustion of the cells. Our data do not show this; rather the calorimeter values are identical for cells grown at all temperatures. The difference in ash content is not viewed as significant. These data seem to suggest that either the molecular changes caused by temperature are small or the cell operates within a caloric parameter. One may look at the data of Tempest and Hunter for *Aerobacter* (33) and make the follow-

**Table 2. Effect of temperature on mass-energy balance in *Pseudomonas fluorescens***

| Temp (°C) | kmax (hr⁻¹) | a (hr⁻¹) | Yield (mg of cells/mg of glucose) | Ash (%) | He (kcal/g) | Efficiency of conversion (%) |
|-----------|-------------|-----------|---------------------------------|---------|-------------|-----------------------------|
| 20        | .250        | .085      | .407                            | 7.5     | 4.86 ± 0.25 | 52.8                        |
| 25        | .420        | .147      | .463                            | 7.4     | 4.85 ± 0.25 | 60.0                        |
| 30        | .630        | .233      | .512                            | 5.4     | 4.80 ± 0.29 | 65.7                        |
| 35        | .240        | .094      | .409                            | 16.2    | 4.94 ± 0.31 | 54.0                        |
ing assumptions: first, that the ash content is a constant 7%, and second, that the lipid is the difference between all constituents and 100%. Thus, we have data for chemical constituents at four temperatures. The heat of combustion of the cells may be estimated by assigning these calorific values: protein, 5.4 kcal per g (35); lipids, 9.3 kcal per g (3); carbohydrates, 4.2 kcal per g (16); and nucleic acids, 3.5 kcal per g (our value for purified ribonucleic acid purchased from Nutritional Biochemicals Corp.). The heat of combustion is calculated as 5.3 kcal per mole at each of four temperatures. Other data which may be similarly calculated are those of Brown and Rose (4) for Candida utilis. For these data, ash may be approximated at 8%, a figure which has been found for nonsporulating yeast (5). At both 15 and 30°C, the heat of combustion is approximately at 5 kcal.

Since the chemical content of the cells is not the same with different temperatures but the heat of combustion is invariant, it appears possible that an energy budget does exist, as shown by the foregoing examples. The question of an energy budget has been raised before by Payne (29). In a most extensive review of yields, Payne found that between 55 and 65% of substrate calories were consistently converted to biomass, irrespective of organism or substrate.

A review of the literature reveals only two cases of heat of combustion being reported for heterotrophs (23, 29), these values being 5.3 and 5.1 kcal per g. However, our values are for steady-state cells and our conditions of pellet preparation differ from those reported.

The expression of Baas-Becking and Parks for the free energy efficiency was modified in view of the criticism of McCarty (19), who pointed out that the expression was derived for chemotrophs, not organisms grown on organic substrates. By substituting the heat of combustion of the substrate for its free energy, the entropy of oxidation is added back (e.g., \( \Delta H = \Delta G + T \Delta S \)), making the expression \( 100 \times (\Delta H_c/\Delta H) \times Y \), a valid one for expressing caloric efficiency in heterotrophs. The data in Table 2 indicate that efficiency of conversion is dependent on temperature.

Since the number of calories per gram (dry weight) of cells is unchanged irrespective of growth temperature whereas the efficiency of conversion is greatly influenced, the prospect of an energy budget becomes more plausible.

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