

Heat of Combustion of Cells of *Pseudomonas fluorescens*

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Based upon 161 determinations, the heat of combustion of cells of *Pseudomonas fluorescens* averaged 5.32 kcal/g on an ash-free dry-weight basis. The standard deviation was 0.39 kcal.

Payne (5) extensively reviewed energy yields and growth of heterotrophs. Heats of combustion of heterotrophs averaged 5.3 kcal/g of dry weight. Payne and his co-workers (2, 3, 6) have themselves determined heats of combustion. In three studies, the average combustion values ranged from 5.27 to 5.38 kcal/g, but the means for individual species differed more widely.

The data reported here are a part of a study of the effect of fluctuation in temperature on bacterial growth characteristics and cell composition (1, 4; R. H. Mennett, Ph.D. thesis, Univ. of Georgia, Athens, 1971; E. Siegler, Ph.D. thesis, Univ. of Georgia, Athens, 1971). Because the temperature of incubation does not seem to influence heat of combustion and this phase will probably not be investigated further, the data are being published to provide a record of the values determined.

Stock cultures of *Pseudomonas fluorescens* were maintained on tryptic soy agar and growth was carried out in tryptic soy broth. For each trial, 2,000 ml of tryptic soy broth was inoculated with a 1% inoculum of an actively multiplying 14.5-h culture. Trials were made at 20, 25, and 30 C and at temperatures fluctuating between 20 to 30 and 30 to 20 C in a sine wave fashion with a period of 90 min. Concurrent studies (1, R. H. Mennett, Ph.D. thesis, Univ. of Georgia, Athens, 1971) established that growth rates of cells are different according to whether cycling is initially upward or downward. All steady-temperature and fluctuating-temperature trials were replicated six or seven times.

Cycling was accomplished by having a Fernbach flask in a 30 C water bath connected by a silicone rubber tube to a second Fernbach flask in an adjacent 20 C water bath. Air was pumped steadily into the inoculated medium. Transfer was controlled by the opening and closing of two

solenoid valves operated by a relay in conjunction with an on-off timer. The air was used both to aerate the medium and to pump the medium from one flask to the other every 45 min. The air going into the flask was sterilized by passing it through a 1.5% solution of HgCl₂. Since this set-up proved to be effective, easy to sterilize, and simple to operate, it is diagrammed in Fig. 1.

Samples for bomb calorimetry were prepared by centrifuging and washing cells from 300 ml of medium, freeze-drying them, and then combusting them in a Phillipson bomb calorimeter. Further details may be found in a previous report (4).

A total of 161 determinations were made for the heat of combustion of cells of *P. fluorescens* (Table 1). The mean value was 5.32 kcal/g. The standard deviation was 0.39 kcal.

Analysis of variance was applied to the values for the five incubation conditions. Cells were harvested at 16, 22, 28, and 40 h of incubation. The F values for temperature and for hours were nonsignificant. When a "t" test was applied between the data for 30 C and that for cycling downward from 30 to 20 C and cycling thereafter in the range 20 to 30 C, there was a statistically significant difference. However, we concur in general with Mennett and Nakayama (4) who concluded that the temperature of incubation in the range 20 to 35 C does not significantly influence the heat of combustion of cells of *P. fluorescens*. Their heats of combustion were lower than ours, but there were differences in the growth conditions. They harvested cells from continuous cultures at the steady state (4) and they used a basic salts-glucose synthetic medium. The medium may have affected cell composition. Prochazka et al. (6) observed a difference between cells of *Clostridium pasteurianum* grown in Trypticase soy broth and in a minimal medium. The respective

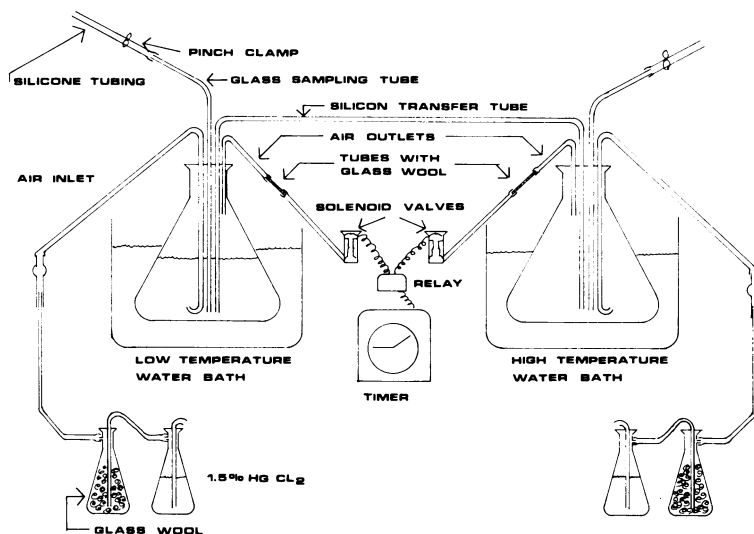


FIG. 1. Apparatus used to cycle inoculated medium in temperature between 20 to 30 C.

TABLE 1. Heat of combustion of cells of *Pseudomonas fluorescens*

Incubation temp (C)	No. determinations ^a	Mean ^b (kcal/g)
20	42	5.46
25	43	5.41
30	43	5.26
20-30	16	5.27
30-20	17	5.48

^a Each determination consists of the mean of duplicate samples and in most cases the mean of triplicate samples.

^b Ash-free, dry-weight basis.

heats of combustion were 5.423 and 4.627 kcal/g. However, the reverse was true when *Pseudomonas C₁₂B* was grown in nutrient broth and a minimal medium.

The difference between our values and those of Mennett and Nakayama (4) is due most likely to the media, as we both used the same bomb calorimeter, the same benzoic acid for instrument calibration, and we followed the

same method of sample preparation except that we redried the cell pellets at 55 C instead of 65 C.

Our data support the conclusion of Payne (5) and others (2, 3, 6) who reported means for microbial cells close to 5.3 kcal/g dry weight.

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