Thermotoga neapolitana: **A microaerophile producing hydrogen in the presence of oxygen**

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Thermotoga neapolitana is an extremophilic bacterium first isolated and described in 1986 as coming from the vicinity of a black smoker in the bay of Naples, Italy [1]. The *Thermotogales* are a ubiquitous order of bacteria and members of that order have been isolated from hot water sources throughout the world. Virtually all of the members of this order are reported to be obligate anaerobes, however, we have examined a number of members of this order and have determined that virtually all of them can tolerate low levels of oxygen. Furthermore, in the presence of oxygen, these bacteria produce hydrogen gas as a byproduct of their metabolism.

We have focused on *Thermotoga neapolitana* quite extensively, and determined that this member of the order *Thermotogales* can metabolize a great variety of different waste carbohydrates (figure 1) and produce exceptionally high concentrations of hydrogen (25-35% v/v). We have optimized pH, temperature, growth medium, and sulfur concentration in an attempt to determine the factors most important for production of high hydrogen concentrations. While all of these factors have some effect, the crucial factor affecting hydrogen production is the concentration of oxygen available in the headspace of the batch reactor at the beginning of the incubation. When starting with a concentration of 6-12% oxygen in the headspace, *T. neapolitana* produces hydrogen and carbon dioxide in a ratio approaching 2:1. Concomittantly, the oxygen originally in the headspace of the batch reactor is depleted. Figure 2 clearly demonstrates this, while one can also see that oxygen is barely utilized if either the substrate or the bacteria are absent in the batch reactor.

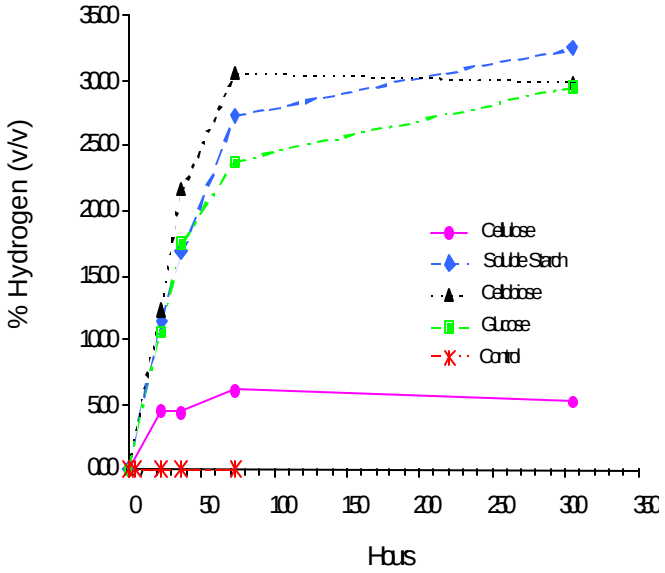


Figure 1: Substrate utilization by *Thermotoga*

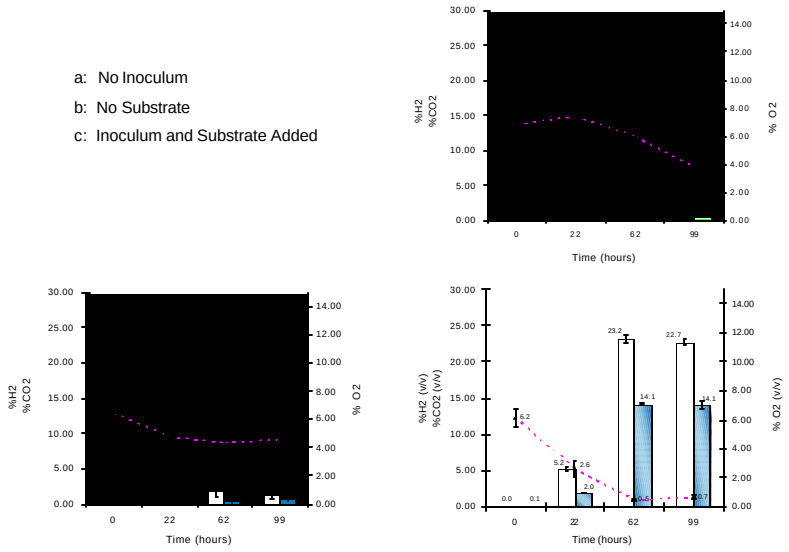


Figure 2: Oxygen utilization and hydrogen production by *Thermotoga neapolitana*

Examination of the efficiency of glucose utilization relative to hydrogen production indicates that even in the logarithmic phase of growth the process can be at least 71 $\pm$ 24 % efficient in terms of the amount of hydrogen produced. However, with a fermentation process the maximum theoretical efficiency [2] is calculated to be between 20-30 percent. Clearly, some other type of metabolic process must be responsible for the greater efficiency, oxygen depletion/utilization, and the 2:1 ratio of hydrogen to carbon dioxide.
We have hypothesized that these bacteria may be microaerophiles. Thus, when they are producing large quantities

of hydrogen, oxygen that is present initially in the batch reactor is depleted/utilized. To determine if this may be a partial microrespiration, malonate, a competitive inhibitor of succinic dehydrogenase, an enzyme of the TCA cycle, was used to inhibit respiratory processes while having only minor effects on fermentative processes (figure 3). The results were specific and dramatic. Figures 4 a shows that normal glucose metabolism and 4b shows metabolism in the presence of a low concentration of malonate. Although fermentative cell population growth can occur in the presence of malonate, hydrogen is not produced until the malonate is bound to the enzme. Thereafter, the bacterial cell population was able to utilize the glucose fully and generate great amounts of hydrogen. An inverse correlation is also seen here between oxygen depletion and hydrogen generation. Most significantly, percent hydrogen does not increase and percent oxygen does not decrease markedly until many hours into the experiment, when it is postulated that all of the malonate would now be bound to the succinic dehydrogenase enzyme and thus no longer be available to inhibit normal glucose catabolism.

These results suggest that *Thermotoga neapolitana* is a microaerophile. In the presence of oxygen concentrations in the headspace of between 6-12 % oxygen, these bacteria are capable of catabolism of glucose and production of hydrogen as a byproduct of carbohydrate catabolism.

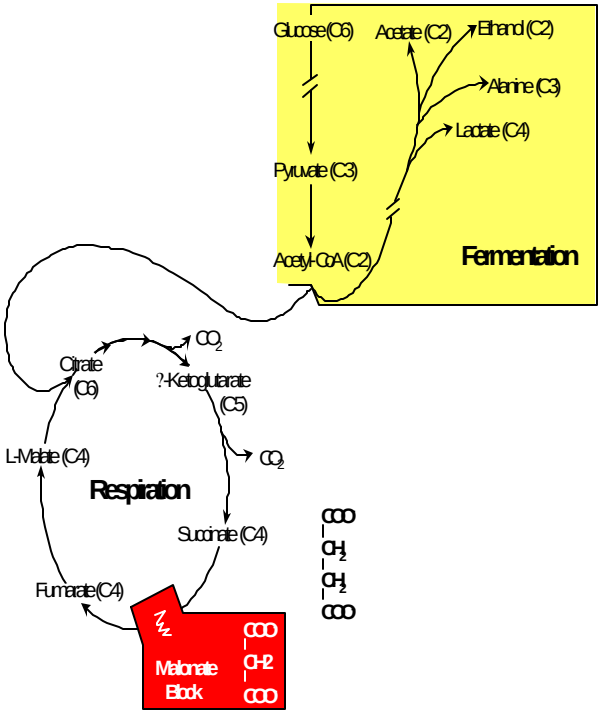
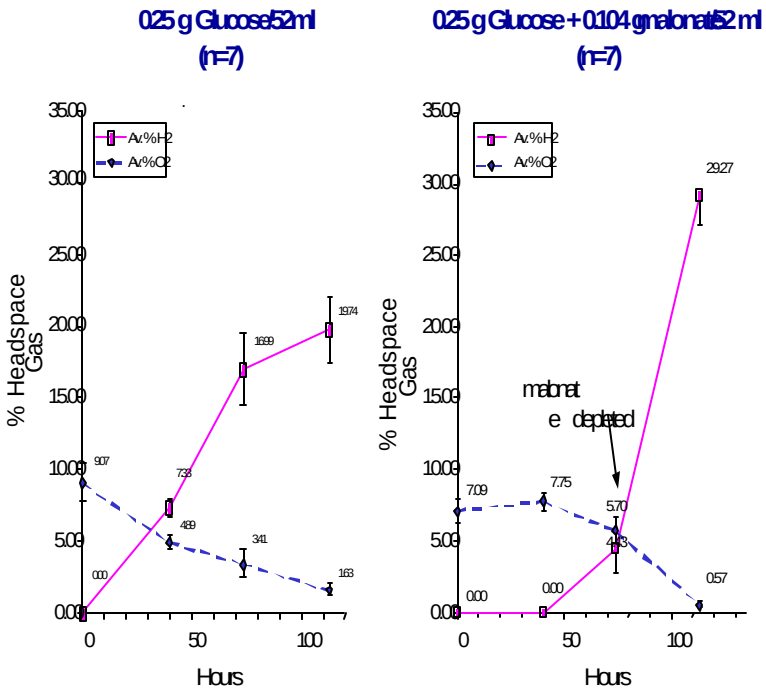


Figure 3: Diagram showing fermentation, respiration, and the site of the malonate. block



Figures 4a & b: Comparison showing oxygen utilization and hydrogen production in the absence (a) and presence (b) of malonate.

1. Jannesh, H.W., R. Huber, S. Belkin, K.O. Stetter 1988 *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*. *Arch. Microbiol.* 150:103-104.

2. Thauer, R.K., K. Jungerman, K. Decker 1977 Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* 41:100-180.