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Microcalorimetric measurements of the activity of a white-rot fungus growing on wood

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Introduction

In a project funded by the Swedish Building Research Council one of the authors (LW) has developed a microcalorimetric method to measure the activity of rot fungi that are growing on wood (Xie et al. 1997). Here we have used this technique to study the activity of a white-rot fungus as a function of temperature.

Material

The white-rot fungus *Collybia maculata*, kindly provided by Dr. Stefan Olsson, Dept. of Microbial Ecology, Lund University, was grown at 25°C in darkness in a Petri dish with malt extract (10 g/l, Merck), agar (15 g/l, Merck) and the polyaromatic dye Poly R-478 (0.2 g/l, Sigma). When the fungus had completely covered the plate, autoclaved (20 min, 121°C) wood sticks of beech (*Fagus sylvatica*, 5x5x20 mm) were put in it. Thereafter, the plate was put in a plastic bag, which was tightly sealed with a TEW Impulse Sealer (O. Möllerström AB, Göteborg, Sweden) and incubated in darkness at 25°C until the wood sticks were completely covered with mycelium. Four well-colonized sticks were used in the present experiments.

Method

The heat production rate (thermal power) at 5, 15, 25, 35 and 45°C was measured in a TAM microcalorimeter (Thermometric AB, Järfälla, Sweden; Suurkuusk and Wadsö 1982). Samples were kept in 3 ml glass ampoules (vials) which were sealed before each measurement. Between the measurements the samples were stored in the unsealed glass vials at 25°C at near 100%RH.

The measurements at different temperatures were made in the microcalorimeter held at the wanted temperatures. Figure 1 shows a typical temperature program from the present measurements. Note that the fungus was only exposed to the measurement temperatures (other than 25°C) during the calorimetric measurements. The duration of most measurements was in the range 7-17 h.

The microcalorimeters are sensitive instruments and one must lower a sample step-wise into the measuring position; otherwise the thermal equilibrium of the instrument will be disturbed. This takes at least 30 min. In the plots, zero on the x-axis is the time a vial was closed and the start point of each curve is the estimated time when the true thermal power from the sample started to be measured.

To measure the heat production from reference samples without living fungi, four samples were used. These samples were previously measured samples that had been inactivated in 100°C for 30 min.

Results

From the reference samples only very low thermal powers, in the order of 0.5 μ W, were measured.

Figures 2-9 shows the calorimetric result of the measurements. Table 1 gives the thermal powers measured after seven hours of measurements for the measurements up to 35°C. As the samples have different sizes we have also scaled the results with the result of the first measurement at 25°C. In this way we decrease the effects of degree of colonization, nutritional status etc. This result is shown in Table 2.

Table 1. The measured thermal powers (μ W) after seven hours of measurement.

	Sample 1	sample 2	sample 3	sample 4
25°C	28.5	29	18	25
25°C (repetition)	27.5	30.5	17	27
15°C	11.5	13.5	6.5	10
5°C	8	8	3.5	7
35°C	38	33	20	33
25°C	13.5	11	8.5	9

Table 2. The mean relative thermal powers measured after 7 hours of measurements at the respective temperature.

25°C	1.00 \pm 0.00
25°C (repetition)	1.01 \pm 0.07
15°C	0.41 \pm 0.04
5°C	0.25 \pm 0.05
35°C	1.23 \pm 0.12
25°C	0.41 \pm 0.07

Discussion

During each measurement at 5, 15 and 25°C the thermal powers were rather constant, but at 35°C they were slightly decreasing. At the last measurement at 25°C the activities were less than half of what they were at the first measurement at 25°C (at the start of the measurement series). This probably means that 35°C damaged the fungus. At 45°C the measured thermal powers start at a high level, but drop down to low values after about four hours. At the following measurement at 25°C only very low thermal powers were measured, so 45°C certainly damaged the fungus.

The 3 ml glass ampoules contained about 2.5 ml of air with 21 μmol of oxygen. If all this oxygen were consumed in an aerobic degradation of glucose, about 9.6 J of heat would be produced. The largest amount of heat measured in any of the present experiments was measured at 45°C when only approx. 2 J of heat was produced; the decrease in activity was thus probably not caused by lack of oxygen. White rot fungi are probably not very sensitive to low levels of oxygen since they usually grow inside wood. The lignin degradation, though, is more efficient at high oxygen concentrations.

Uses of the result

Modeling fungal activity as a function of temperature

We have measured the relative heat production of a fungus in the range 5–45°C. Similar measurements have also been done on six other rot fungi (Bjurman and Wadsö). From this material it is possible to model the rate of biodeterioration as a function of temperature if we assume that the growth of the fungi is only a function of temperature. Let us define the relative thermal power $p(T)$ as has been done in Table 1:

$$p(T) = \frac{P(T)}{P_o} \quad (1)$$

Here P_o is the thermal power measured at a reference temperature; 25°C in the present case. If we assume that only the temperature governs the rate of degradation (i.e. moisture content and oxygen concentration are not rate limiting) the degradation D (that may have units of g/g, i.e. D is the fraction of a material consumed) during a time period may then be written:

$$D = f \int_{t=0}^t p(T(t)) dt \quad (2)$$

Here f (unit: g/g/s) is a usually unknown factor that is dependent on the amount of fungus (we also assumed that the temperature changes are so slow that we do not have any transient effects). From this it is seen that it is difficult or impossible to calculate the actual damage, but it is possible to compare the degradation during different temperature schemes.

In the following we will use the relative thermal power p as a function of temperature as a measure of fungal activity and give some practical examples of what it may be used for.

Hazardous evaluation

Assessment of the risk of self-ignition is a problem wherever large amounts of materials or substances are stored. With rot fungi the largest problems are probably with long-term storage of wet biological fuels (wood particles, peat) but the same principles apply to mold fungi that may grow at much lower water activity.

Consider a 5x5x5 m³ pile of wood chips infected with rot fungi. Let us assume that the heat produced in the pile is only a function of temperature (i.e. moisture and oxygen concentrations high enough not to be rate limiting). The present data (measured on approx. 1 ml of material) gives that at 5 and 25°C the pile would produce 150 and more than 600 W, respectively. In the outer parts of the pile the heat may be lost to the surroundings, but the heat produced in the center will tend to increase the temperature in the pile. Consequently the temperature will rise, causing other microbiological and chemical processes to start, and in the end the material may

be more or less consumed by the processes or even self-ignite. The path of the process may be highly dependent on the microorganisms present in the material. Fresh saw dust from a sawmill may contain much fewer microorganisms than a bio-fuel that has been produced in the forest and then transported, reloaded etc.

Degradation with rot fungi

White-rot fungi may in some cases break down toxic substances in the soil. In the ground the situation is quite complex as moisture state, oxygen concentration, temperature and the chemistry of soil and substrate (that the fungi is living on) may influence the fungal activity. *If* we assume that the rate of the degradation process is proportional to the metabolic rate of the fungi, then the present type of data may be used to calculate the relative rate of the process as a function of the climatic conditions and the depth in the ground as the soil temperature over a year is dependent on depth in the ground.

The microcalorimetric technique may be developed so that one may vary more factors than the temperature. It is probably possible to incorporate a tension plate device in the calorimeter to generate water potentials between -1 atm and 0. This would cover most situations found in natural soils. If lower water potentials are needed a pressure plate has to be used, but this has the disadvantage of exposing the soil sample with the fungus to high pressures that may influence the fungal activity. The oxygen levels may be controlled in a sample in the microcalorimeter by perfusion of gas through the calorimetric ampoule.

References

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Suurkuusk, J. and Wadsö, I. (1982), "A multichannel microcalorimetry system", *Chemica Scripta*, 20 253-263

Xie, Y., Bjurman, J. and Wadsö, L. (1997), "Microcalorimetric characterization of the recovery of a brown-rot fungus after exposures to high and low temperatures, oxygen depletion and drying", *Holzforschung* 51 201-206.

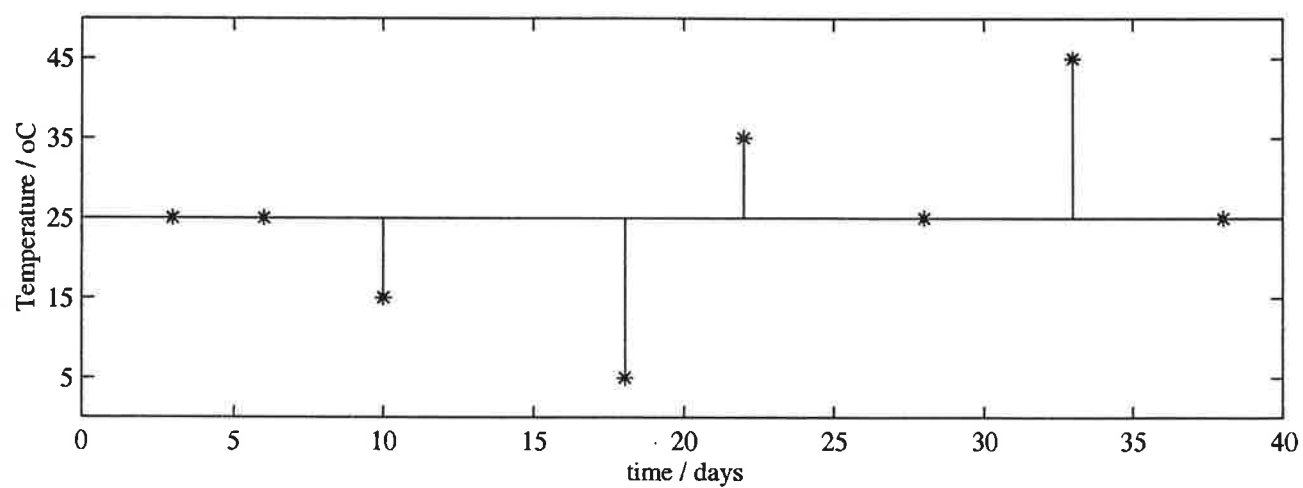


Figure 1. A typical temperature program. The samples were held at 25°C except during the measurements (stars).

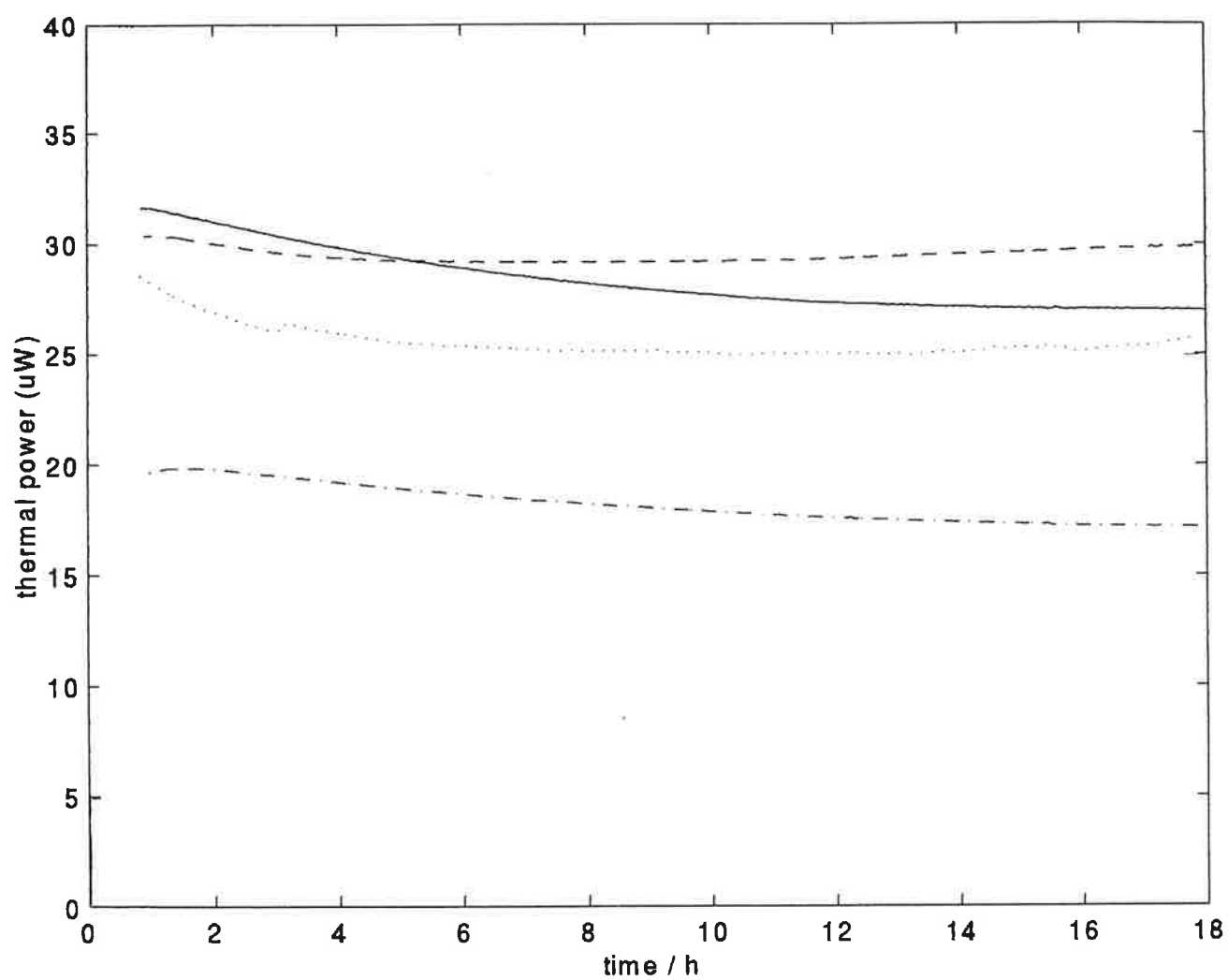


Figure 2. Result of first measurement at 25 °C. Sample 1 (solid line), sample 2 (dashed line), sample 3 (dash-dotted line), and sample 4 (dotted line).

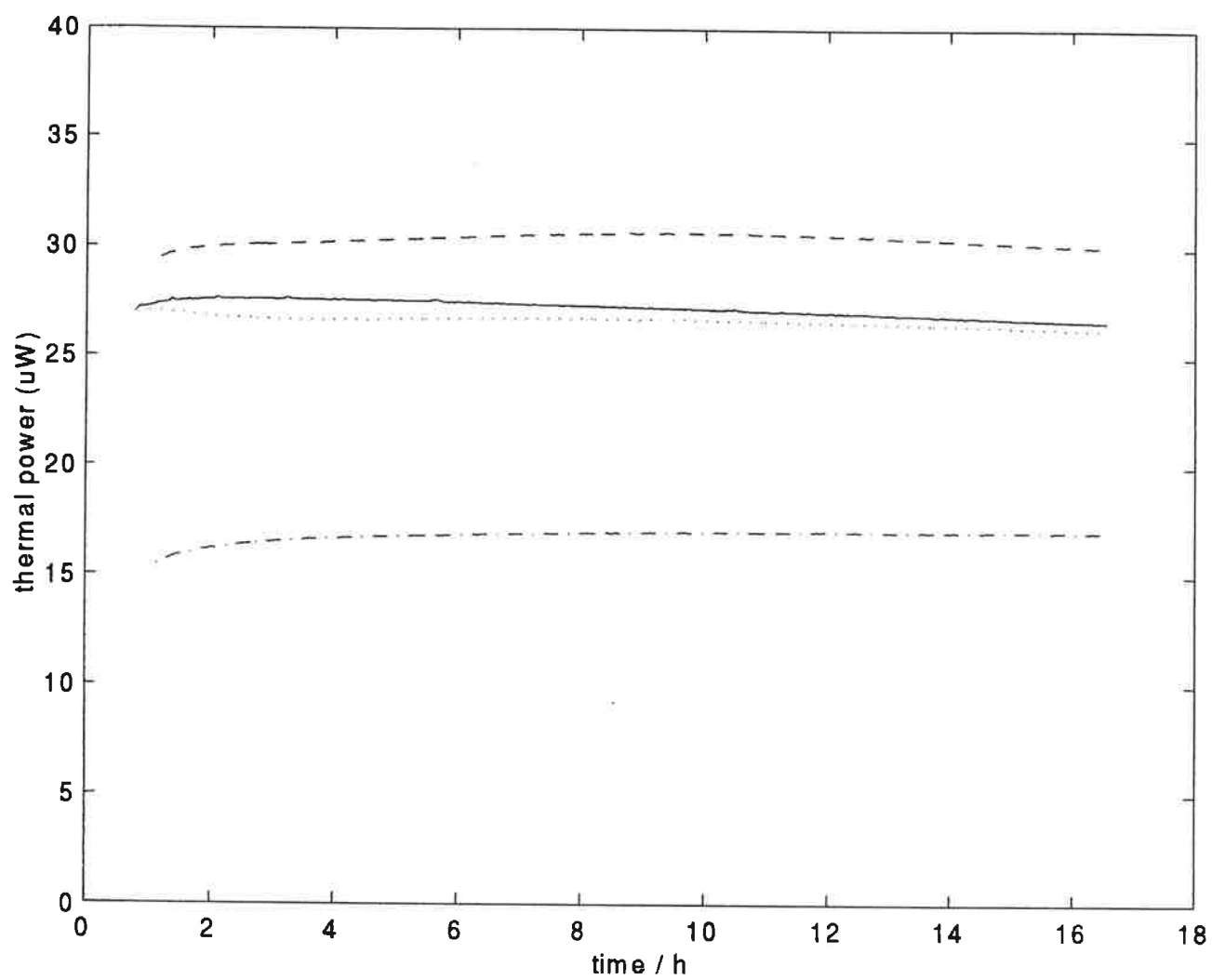


Figure 3. Result of second measurement at 25 °C. The same legends as in Fig. 2.

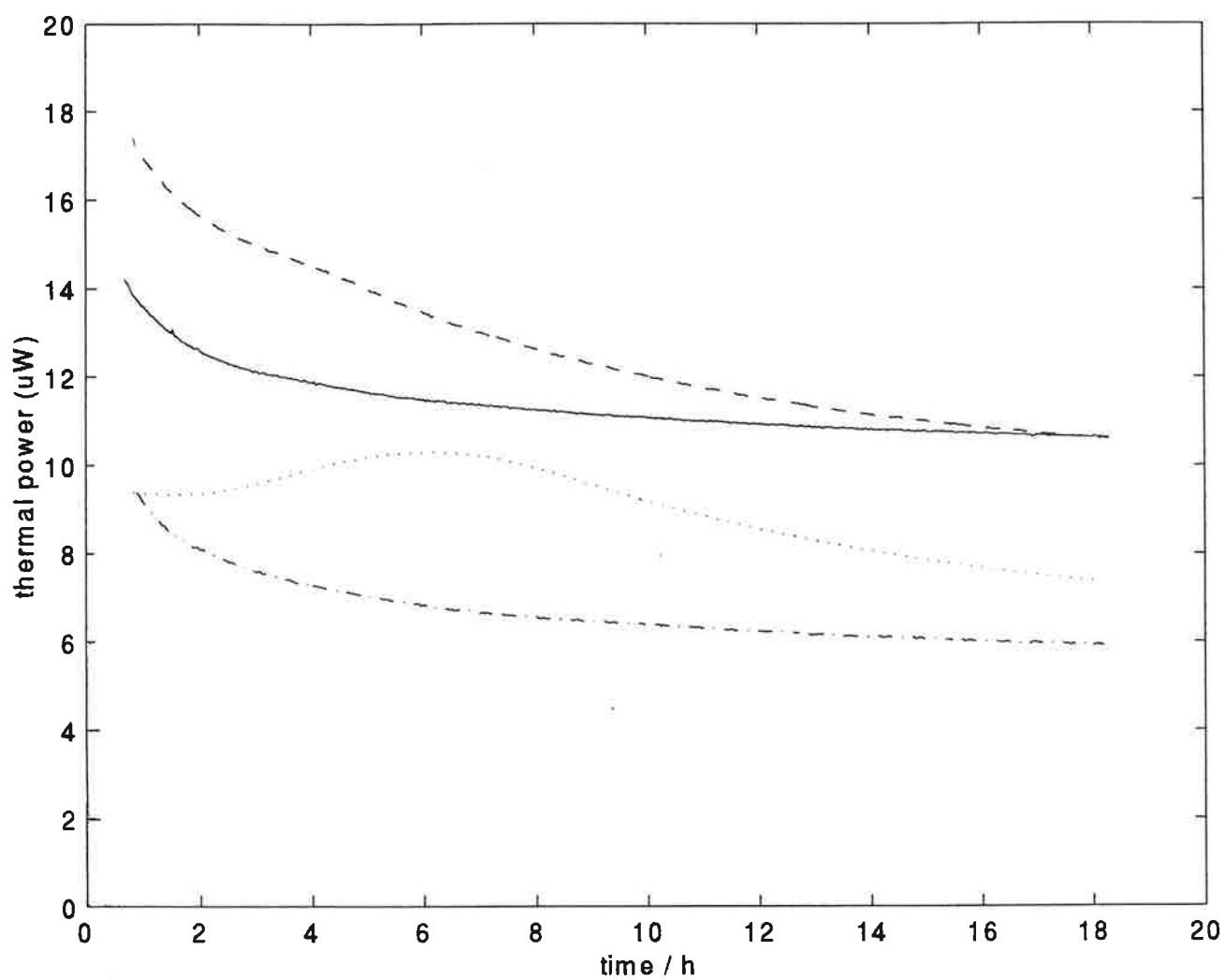


Figure 4. Result of measurement at 15 °C. The same legends as in Fig. 2.

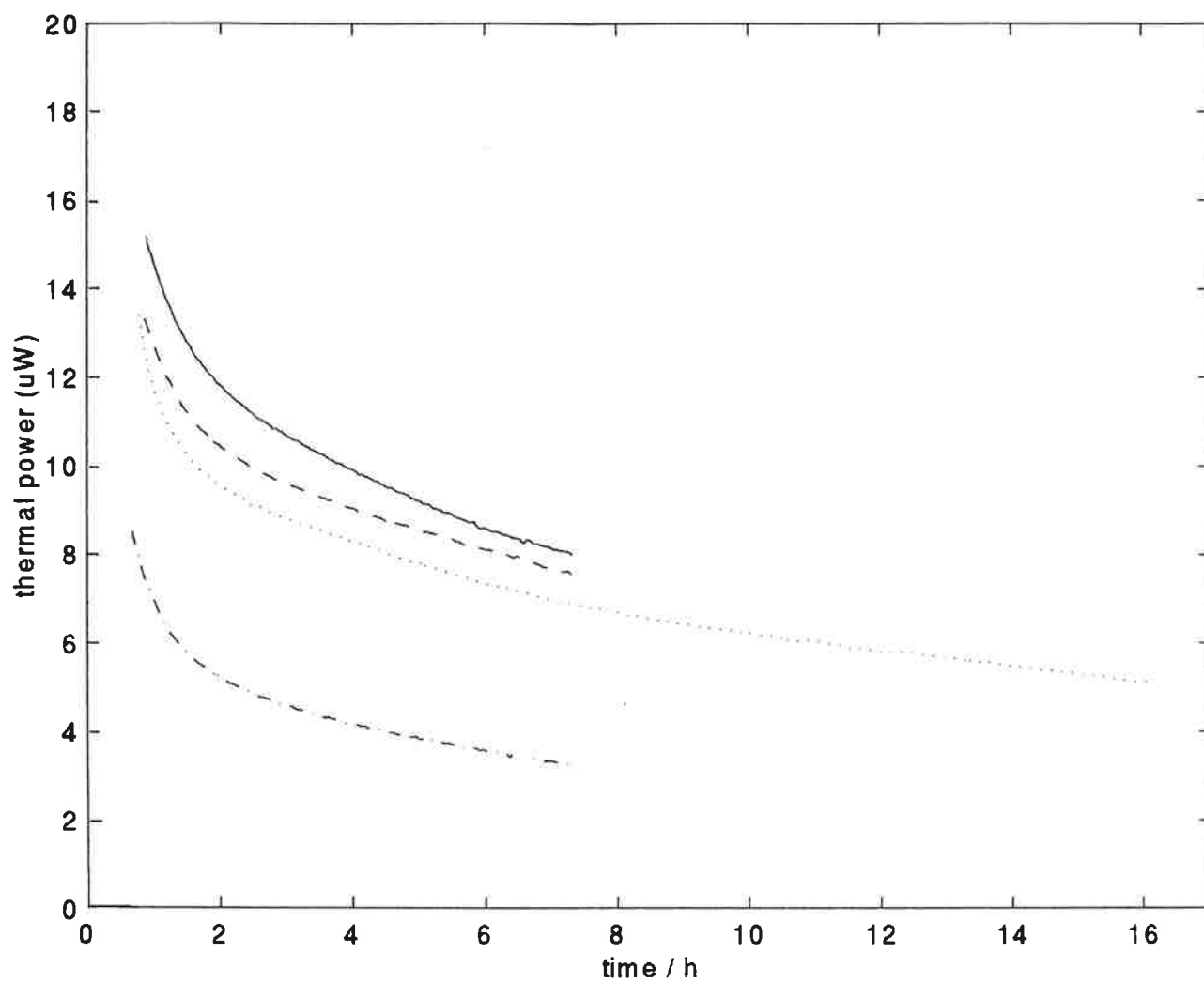


Figure 5. Result of measurement at 5 °C. The same legends as in Fig. 2.

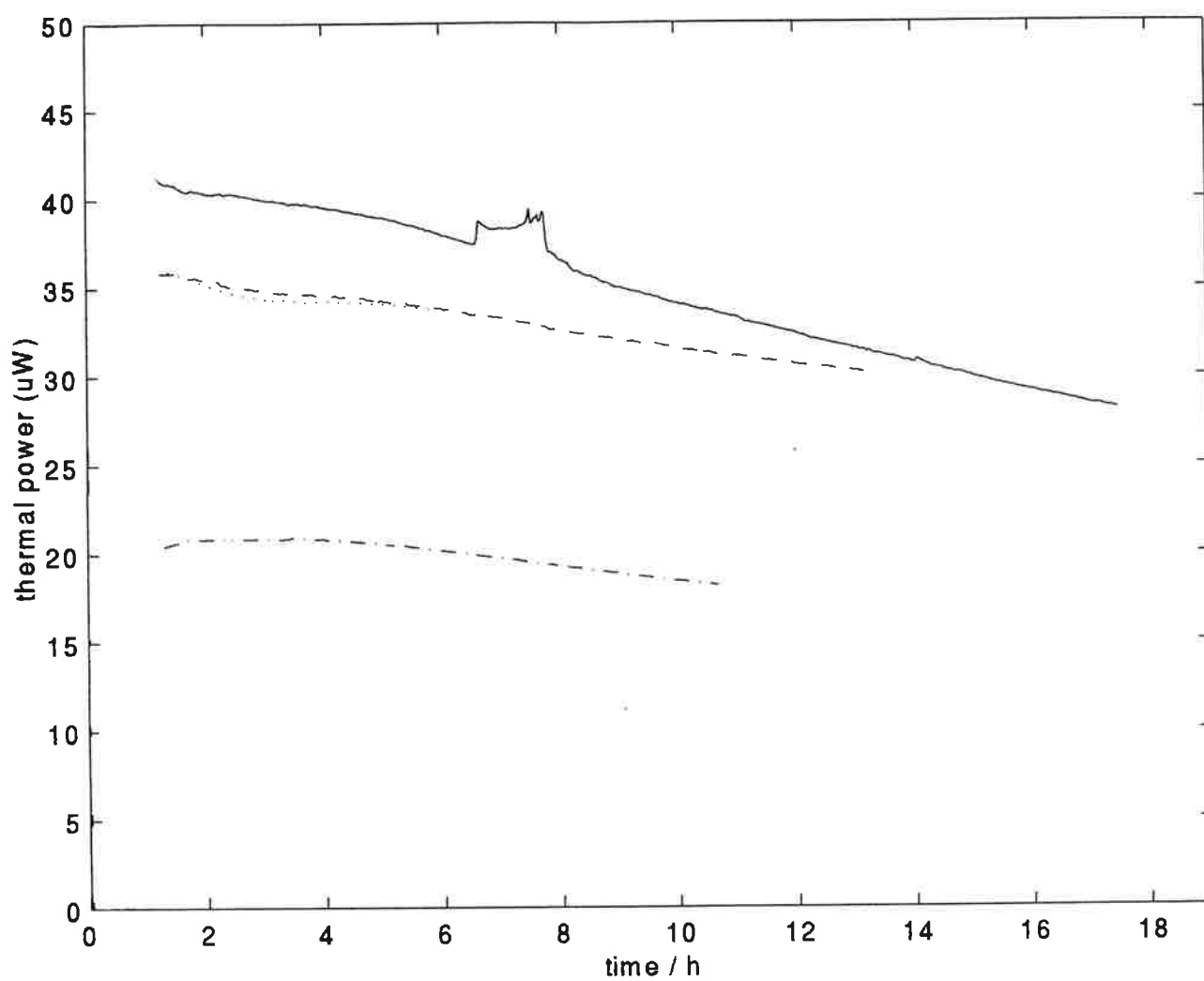


Figure 6. Result of measurement at 35°C. The noise seen is probably an electrical artifact and not a result of fungal activity. The same legends as in Fig. 2.

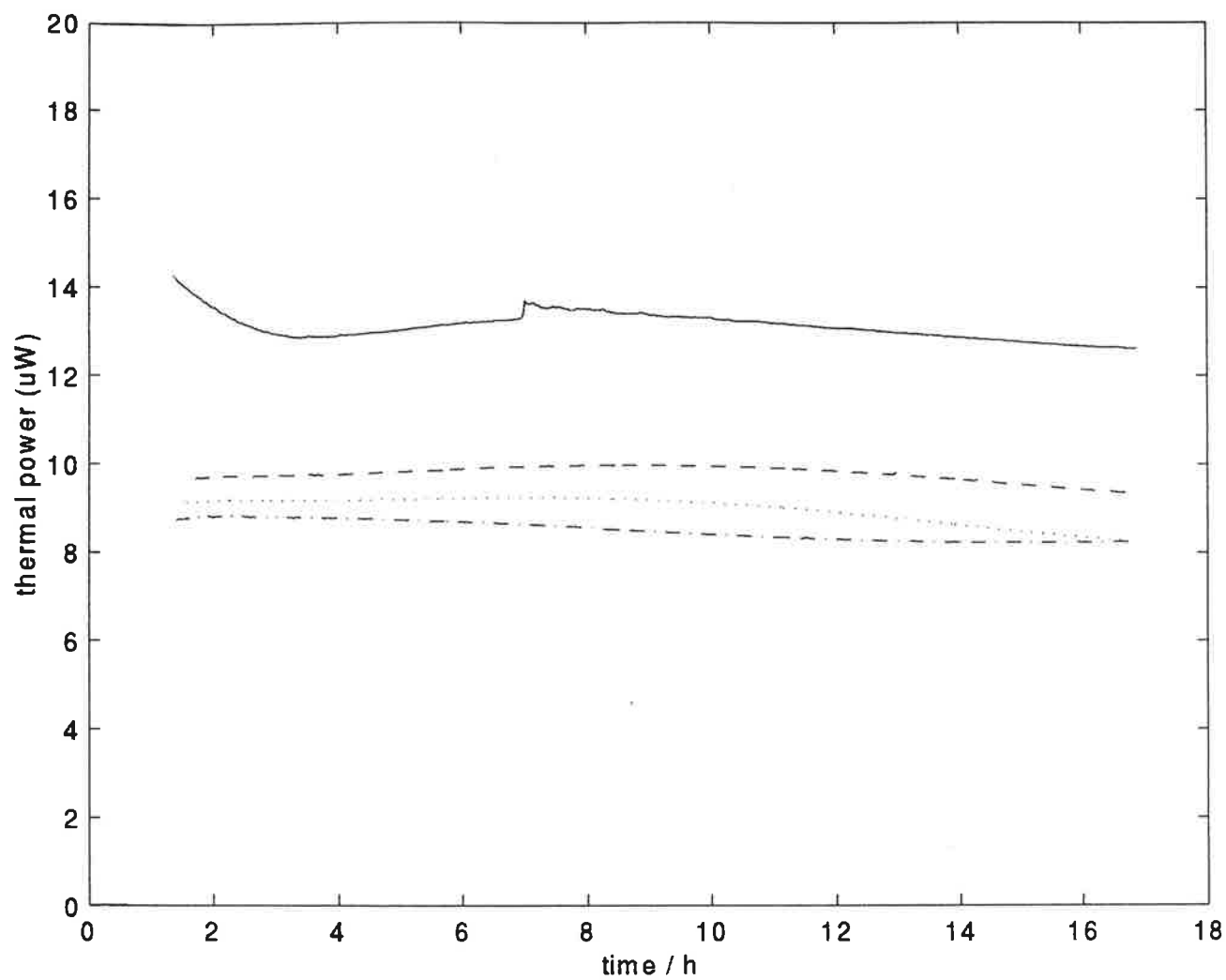


Figure 7. Result of measurement at 25 °C after the measurement at 35 °C. The noise seen is probably an electrical artifact and not a result of fungal activity. The same legends as in Fig. 2.

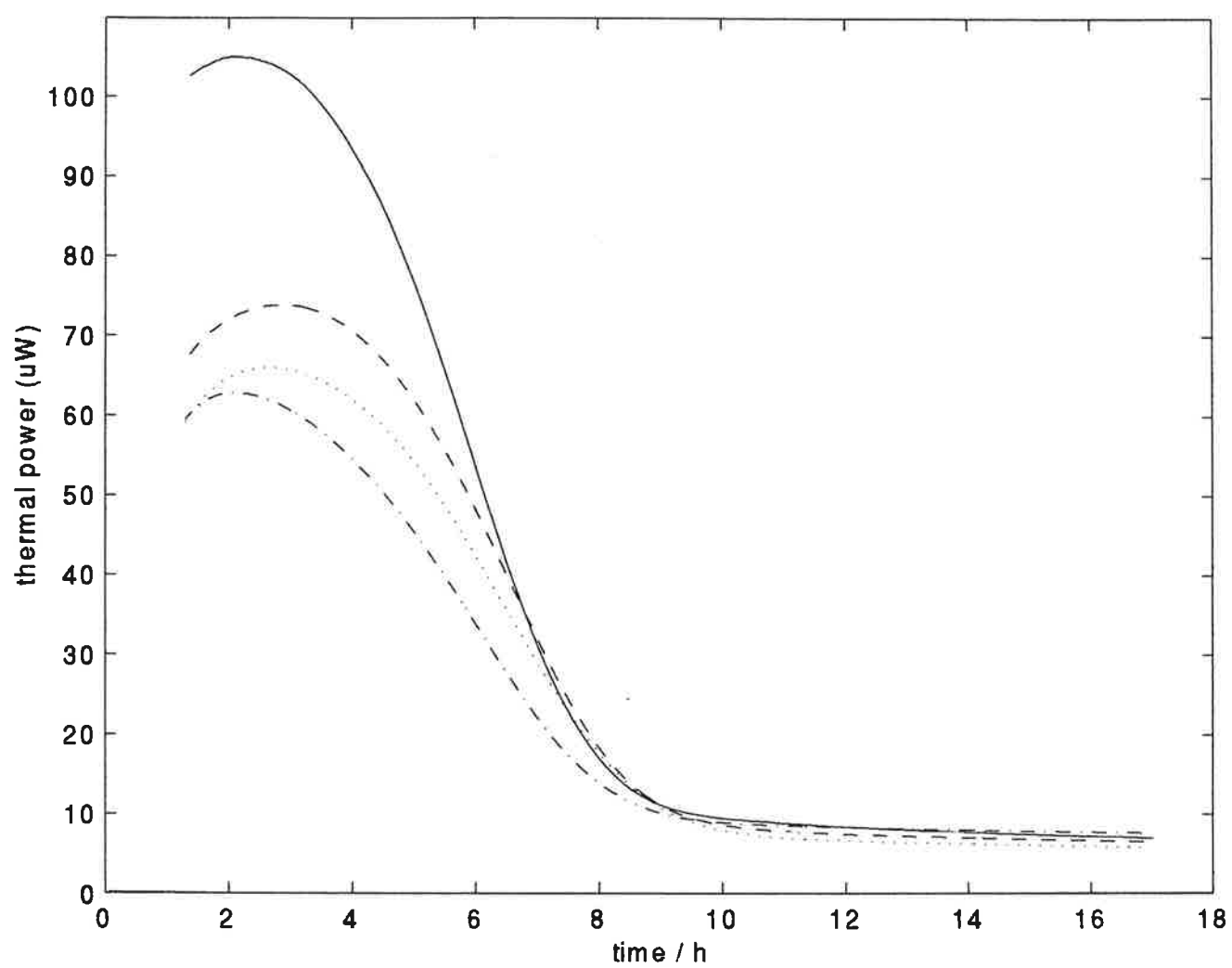


Figure 8. Result of measurement at 45 °C. The same legends as in Fig. 2.

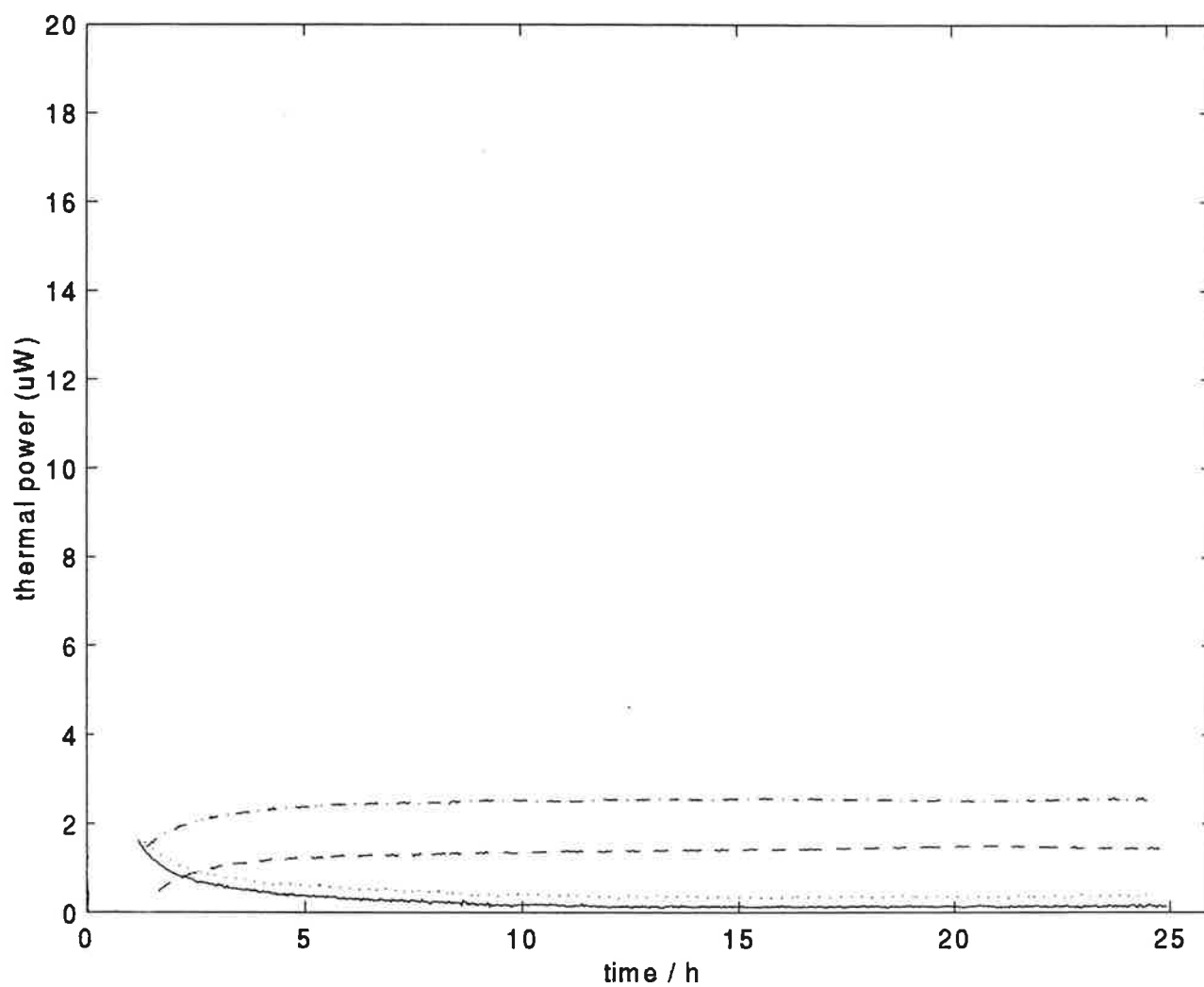


Figure 9. Result of measurement at 25°C after the measurement at 45°C . The same legends as in Fig. 2.