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Correlating two methods of quantifying fungal activity: Heat production by isothermal calorimetry and ergosterol amount by gas chromatography–tandem mass spectrometry

Yujing Li [a,*], Lars Wadsö [a], Lennart Larsson [b], Jonny Bjurman [c]

[a] *Division of Building Materials, Lund University, Box 118, SE 221 00, Lund, Sweden*
[b] *Department of Laboratory Medicine, Lund University, Sölvegatan 23, SE 223 62 Lund, Sweden*
[c] *Department of Conservation, Göteborg University, Box 130, SE 405 30 Göteborg, Sweden*

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Abstract

Two methods of quantifying fungal activity have been compared and correlated: isothermal calorimetry for measuring heat production and gas chromatography–tandem mass spectrometry (GC–MS/MS) for measuring ergosterol, a proxy for biomass. The measurements were made on four different fungi: *Penicillium roqueforti*, *Cladosporium cladosporioides*, *Neopetromyces muricatus* and the dry rot fungus *Serpula lacrymans*. The results showed linear correlations between ergosterol production and total heat production for these four fungal species during the initial fast growing stage. At the later stages heat was produced but ergosterol amount was constant. The heat produced per ergosterol amount varied from species to species and between different temperatures. This might be due to the different metabolic efficiencies of different species or the same species at different temperatures. Isothermal calorimetry can be used in fungal studies on its own or in combination with other techniques for a more complete understanding of fungal physiology.

Keywords: Isothermal calorimetry; Gas chromatography–tandem mass spectrometry; GC–MS/MS; Fungal activity; Heat; Ergosterol

1. Introduction

Measurements of fungal activity (here the word activity is used as a general term) can be made in different ways depending on the aim of a study and the methods available. Methods for such studies can be roughly classified into four groups according to which aspects of the fungi one focuses on:

(1) Biomass estimation methods such as measurements of dry mass of mycelium, or contents of specific compounds like ergosterol [1,2], ATP concentration [3] or chitin [4,5].
(2) Growth rate estimation methods such as rate of hyphal elongation [6], respiration [6,7] and heat production rate [8].
(3) Bio-marker methods, i.e., the measurement of certain fungal products such as spores [9] or mycotoxins [10].
(4) Indirect methods such as weight loss of solid substrates like wood [11].

Group 1 includes those methods that measure the amount of fungi, and group 2 includes those that measure the rate of change of the amount of fungi (growth). Groups 3 and 4 methods measure other events that are not expected to be directly related to biomass or growth, but more related to the impact of the fungal growth such as production of toxic compounds and degradation of timber.

It is obvious that the above mentioned methods measure different aspects of fungal activity. For example, measurements of hyphal elongation of a filamentous fungus will give an index of the biomass accumulation, whereas the amount of mycotoxin is generally not proportional to biomass, but much dependent on environmental conditions. Results from different methods may thus look quite different but are not necessarily in conflict. Note also that even quite different methods are often not totally independent from each other. For example, biomass accumulation cannot be achieved without metabolism.

It is not possible to generally say that one method is better than any other since different methods look at different aspects of the state of a fungus. All methods have their advantages and limitations. For example, biomass estimation methods are accurate, but destructive. Thus, such a method can be used one time

* Corresponding author. Tel.: +46 46 2229222.
E-mail address: yujing.li@byggtek.lth.se (Y. Li).

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but not as a continuous measurement. The use of more than one method will give more information than will any of the methods alone, and investigations of how the results of different methods correlate can be a valuable way to obtain a more complete picture of fungal growth.

Isothermal calorimetry measures thermal power (W; heat production rate or – more informally – thermal activity) and heat at constant temperature. Heat (J) is the integral of the thermal power–time curve. It has been used in biology since 1770s when Adair Crawford studied animal heat [12]. A few years later Lavoisier and La Place made their remarkable discovery of the similarity of chemical combustion and biological respiration [13]. The thermal power measured from biological samples is produced by the biochemical reactions of their metabolism. Over the years, more and more sensitive calorimeters have been used in the biological field to study "from macromolecules to man" [14]. Some examples of organisms studied are germinating seeds [15], bacteria [16], yeast cells [17,18], plant tissue [19,20], thermogenic flowers [21], vegetable tissue [19], human cells [22], mammalian cells [23], insects [24] and fish [25]. Except measurements on yeast and complex samples such as soils and foodstuffs that may contain fungi, there have been only a few calorimetric studies done on fungi. Xie et al. [8] and Bjurman and Wadsö [26] studied rot fungi growing on wood, Markova and Wadsö [27] and Wadsö [28] measured mould activity as a function of water activity and Wadsö et al. [29] made a calorespirometric study of two *Penicillium* species.

The heat production rate (thermal power) measured from fungi reflects the fungal metabolic activity. However, how the thermal power is related to other physiological aspects of fungi is still unknown. Therefore, it is meaningful to correlate this method to accepted methods in fungal studies. Besides, isothermal calorimetry might also give additional information compared to the available methods. Therefore, the aim of the present study is to compare and correlate two quite different methods to quantify fungal activity: heat production by isothermal calorimetry and amount of ergosterol determined by gas chromatography–tandem mass spectrometry (GC–MS/MS).

Measurements with isothermal (heat conduction) calorimeters can be performed in many different ways; in its simplest form (which has been used in the present work) samples are placed in sealed vials resting on heat flow sensors in contact with extremely well-thermostated heat sinks. Vial sizes are typically 0.5–20 ml. A general trend in isothermal calorimetry has been towards more and more sensitive instruments. Today, much microbiological work is done with microcalorimeters that have baseline sensitivities in the order of 1 μW. However, increased sensitivity normally limits the volume and numbers of samples that can be measured simultaneously due to the sensitivity and complexity of the instruments. In the present study, we have worked with a slightly less sensitive instrument that has the advantage of containing eight separate calorimeters, therefore, many measurements can be performed in parallel.

Ergosterol quantification is an established method in fungal research [30–33]. Ergosterol is a sterol that is found almost solely in the cell membranes of fungi [1,4,34]. It is thus well suited to be a unique chemical marker in fungal biomass estimation. Ergosterol determination by HPLC with a UV detector was first introduced by Seitz et al. [34] as a measure of fungal growth in grain. Since then ergosterol analysis has been used in many studies [35] and different variants have been developed with or without a hydrolysis step.

The content of ergosterol in fungal mass is rather constant. Newell found a mean ergosterol content of 6.2 mg/g mycelial mass with only 8% coefficients of variation [1]. This mean value is close to the 5 mg/g value suggested by other researchers [36,37]. Newell concluded that "ergosterol values can be converted into fungal mass values without the risk of large error due to variation in conversion factors" [1]. However, some studies have shown that the amount of ergosterol can be dependent on species [38,39]. Studies have also shown that environmental factors can influence the ergosterol content: it was lower in mycelia growing on low nutrient media and after moisture stress [40,41], and it decreased in older cultures and after reduced aeration [42]. Even during short term experiments in surface liquid cultures the conversion factor for cultures of *P. brevi-compactum* varied from 2 to 10 mg/g dry weight of mycelia in one study [40]. However, estimation of ergosterol amount is still well accepted as a reliable method to detect and quantify the existence of fungi in mycological and environmental studies. As mentioned above, a conversion factor of 5 mg ergosterol/g dry biomass is common in fungal research.

In the present study, we use isothermal calorimetry together with quantification of ergosterol by GC–MS/MS in order to investigate if there is a correlation between the heat production and amount of ergosterol. This is the first study of this type.

2. Experimental

2.1. Materials

Three mould fungi and one rot fungus have been investigated: *Penicillium roqueforti*, *Cladosporium cladosporioides*, *Neopetromyces muricatus* and *Serpula lacrymans* (Table 1). They were grown on 2% malt extract agar (MEA) (Merck, Germany) substrate before inoculation.

2.2. Sample preparation

Fungal samples were inoculated from fresh fungal colonies into 20 ml calorimetric glass vials (Thermometric AB, Järfälla, Sweden; inner diameter about 25 mm) containing 2 ml of 2% MEA substrates with a water activity close to 1.0. Between the calorimetric measurements throughout the experimental period, the vials were kept at constant temperature (Table 1) in a large closed container with water on the bottom to provide with a relative humidity above 97% as measured by a capacitive relative humidity probe. To prevent contamination of the samples while making gas exchange possible, humidified cotton plugs were placed in the opening of the vials. At regular intervals about three samples were removed from the experimental group and placed in a low temperature freezer ($< -20\,^{\circ}C$) for further ergosterol quantification. Low temperature was used to prevent

Table 1
Overview of measurements and some results. The heat per ergosterol was calculated from the initial growing stage and the maximal amount of ergosterol in the vials was calculated from the final stage

Experimental group	Species	Temperature (°C)	Number of samples	Ergosterol (J/μg)	Maximum mass ergosterol (μg)
1	*Serpula lacrymans*[a]	20	32	5.4	55
2	*Penicillium roqueforti*[b]	20	40	0.8	55
3	*P. roqueforti*[b]	15	48	3.0	50
4	*Cladosporium cladosporioides*[c]	15	37	1.8	50
5	*Neopetromyces muricatus*[d]	15	40	4.2	40

[a] Strain SL1 from Haavard Kauserud, Oslo University, Norway.
[b] Strain from Biocentrum, Technical University of Denmark.
[c] Strain CC1, isolated from wood in a house in Skåne in the southernmost part of Sweden.
[d] Strain NM1, former *Aspergillus muricatus*, isolated from wood in a house in Skåne in the southernmost part of Sweden.

further growth and ergosterol degradation. Glass vials with only MEA agar substrate but no inoculums were used as controls.

2.3. Calorimetry

A TAM Air (Thermometric AB) instrument with eight separate isothermal calorimeters was used. Each calorimeter was electrically calibrated. The sample vials were removed from the container and sealed with aluminium caps with Teflon-rubber septa and placed in the isothermal calorimeter. Measurements were performed soon after inoculation and thereafter, every second day at 15 or 20 °C in accordance with the growth temperature of the experimental group.

After 30–60 min of equilibration, a steady state was achieved for each measurement. The value at 90 min from the start of the calorimetric experiment was used for evaluation. A thermal power–time curve was constructed with the data obtained from each measurement of a given sample which was further integrated to obtain the value of the heat produced from inoculation until the end (the sample was frozen) of the experiment. This is illustrated in Fig. 1 for one of the samples from each measurement group.

2.4. Ergosterol

Ergosterol was quantified at the Section of Medical Microbiology, Lund University, as described previously [43]. In brief, samples were heated in 10% methanolic KOH at 80 °C for 90 min and partitioned twice with heptane–water (1:1, v/v). The combined heptane phases were evaporated to dryness, dissolved in 1 ml of heptane–dichloromethane (1:1, v/v) and purified using a disposable silica gel column. Derivatisation was performed by heating in BSTFA (50 μl) and pyridine (5 μl) at 60 °C for 30 min. Heptane (50 μl) was added to each preparation prior to analysis by using gas chromatography–tandem mass spectrometry; quantification was performed by using dehydrocholesterol as an internal standard [43].

3. Results and discussion

There was no visible colony on the agar surface during the first days after inoculation. Colonies spread out from the inoculum point until they covered the surface of the agar media in the vials. Thereafter, there was no change in the appearance of the fungal colonies. The colours of the colonies were as follows: *S. lacrymans*, white–ivory; *P. roqueforti*, green; *C. cladosporioides*, dark brown–black; *N. muricatus*, yellow–light brown. The samples in the same group were similar in colour, shape and size development.

The thermal activity of the fungal samples showed similar accelerating and declining phases for all five experimental groups as shown in Fig. 1 (upper row). There was no or little thermal activity measured during the first days after inoculation. Then, the thermal activity of the fungal samples started to

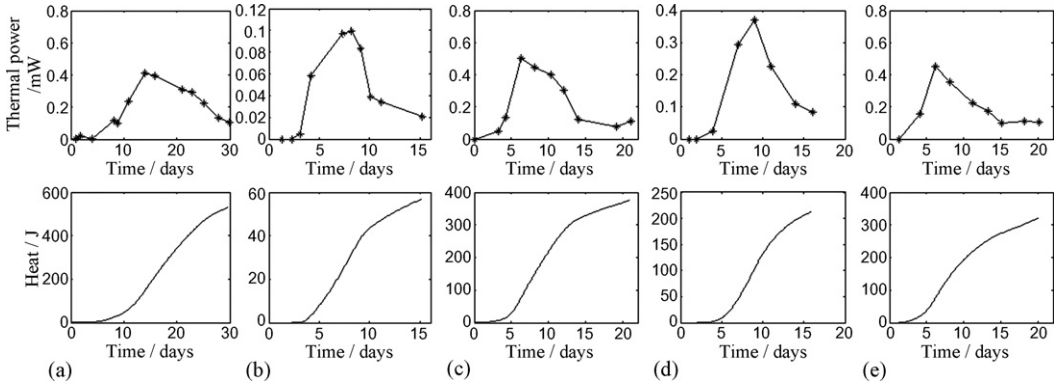

Fig. 1. Upper row: power–time curves obtained after linear interpolation of the calorimetric data measured during 20–30 days for a given sample. Bottom row: heat–time curves obtained after integration of the curves in the upper row. (a) *S. lacrymans*, 20 °C; (b) *P. roqueforti*, 20 °C; (c) *P. roqueforti*, 15 °C; (d) *C. cladosporioides*, 15 °C; (e) *N. muricatus*, 15 °C.

increase until their peak levels after 7–10 days. After this, the thermal activity went down. This decline may have been caused by lack of nutrients or by space limitation when the colonies reached the walls of the vials.

Fig. 2I, curves a–e show the total heat produced in all the samples of the five experiment groups. Their corresponding ergosterol contents are shown in Fig. 2II, curves a–e. The Gompertz function [44] was used to fit both the heat and the ergosterol results:

$$N = A\ \exp(B\ \exp(-\exp(C - Dt))) \quad (1)$$

where N is the heat or ergosterol produced, t the time and constants A–D are the fitting parameters. The Gompertz function was originally suggested by Gompertz [45] as an empirical equation expressing human mortality data, but has later been

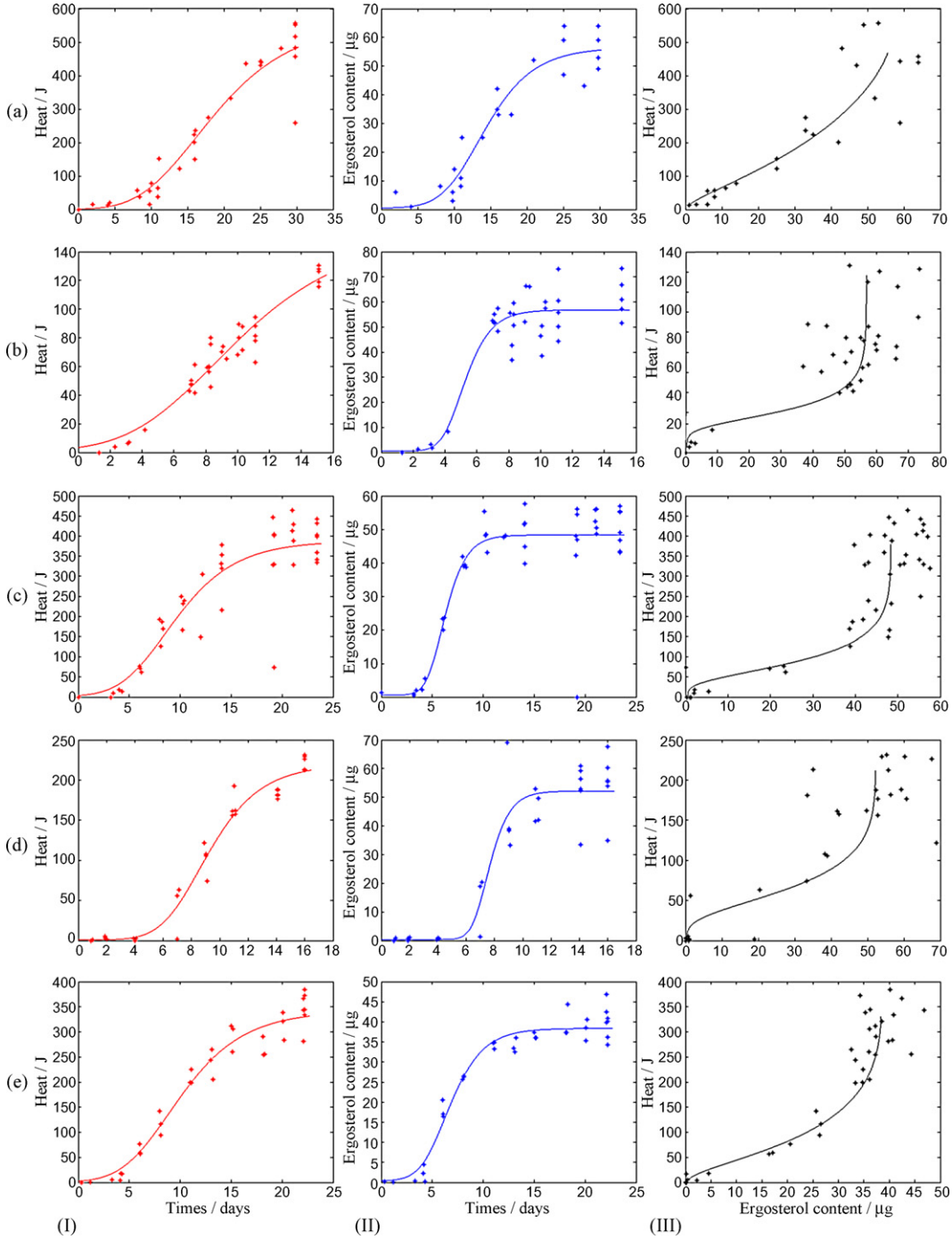

Fig. 2. (I) Total heat produced during fungal growth with Gompertz fitting curve; (II) ergosterol content of fungi during growth and Gompertz fitting curve; (III) total heat production vs. ergosterol content during fungal growth. (a) *S. lacrymans*; (b) *P. roqueforti* (20 °C); (c) *P. roqueforti* (15 °C); (d) *C. cladosporioides*; (e) *N. muricatus*.

frequently used to fit microbiological growth curves. Although the fitting parameters have later been given physical interpretations [44] its use in this work is only as a convenient fitting tool. It is seen that for the groups the fit for the ergosterol curve ends at constant levels, but the fit for the heat curve still increases. Fig. 2III, curves a–e correlates heat production with ergosterol content. Initially, there is a relatively linear trend in heat as a function of ergosterol content. From the initial rather linear slopes of the lines in Fig. 2III the amount of heat produced per mass of ergosterol for different strains were calculated to be 0.8–5.4 J/μg (Table 1).

An interesting feature that arises from Table 1 is the similar final ergosterol content for every strain studied (about 50 μg). This suggests that the total amount of ergosterol or total amount of biomass production is related to the total amount of nutrients provided in the substrate. However, the heat production continues when the ergosterol level stays constant. This phase coincides with the maintenance phase in classical microorganisms growing curves. There may still be nutrients left and then some other factor must limit the ergosterol level, or the fungi utilize its own nutrients stored in hypha to keep alive. Unfortunately, we do not know the nutritional value of the malt extract agar for the present organisms and have not measured nutrient concentrations during the experiment. One complication is also that fungi may break down old parts of their hypha and reuse at the growing tip [46]. This has been indicated also in relation to ergosterol measurements of decaying wood [47]. If this is the case the heat–ergosterol relation in older samples may be very complex.

The coefficients of variation (CV) for heat and ergosterol determinations were about 12 and 10%, respectively (calculated from the latter measurements for series c; cf. Fig. 2). For the heat determinations the largest source of error is probably the integration of a limited number of data points (cf. Fig. 1). Baseline levels are also a source of uncertainty. For the ergosterol measurements, the main source of variation is the GC–MS/MS method that in a method study [43] had a CV of about 7%. The final ergosterol levels may also have been influenced by the variation in the volume of substrate (CV less than 5%).

Moulds such as *P. roqueforti* are aerobic organisms (most fungi are aerobic; a notable exception is anaerobic rumen fungi [4] and some yeasts – sometimes defined as fungi – are facultative anaerobes [48]). If we accept that fungi contain 5 mg ergosterol/g biomass, the 800 and 3000 J/mg ergosterol measured at 20 and 15 °C, respectively, are equivalent to 4.0 and 15 J/g produced biomass.

The aerobic metabolic reactions can conceptually be divided into two parts: catabolic and anabolic. The catabolic reactions consume oxygen and substrate to produce carbon dioxide and energy that drives the anabolic reactions (that may also involve oxygen and carbon dioxide). Catabolic processes generally have significantly higher enthalpies (per mol C) than do anabolic processes [49].

The substrate carbon conversion efficiency is the fraction of the carbon in the consumed substrate that ends up as biomass. If the catabolic and anabolic processes of an aerobic organism do not change qualitatively when the temperature changes, an increase in produced heat per produced biomass must be caused by a relative increase in the rate of catabolic processes relative to the rate of the anabolic processes. This indicates that the increase in heat per biomass seen as the temperature is decreased is the result of lowered substrate carbon conversion efficiency. However, it is also possible (or even probable) that the catabolic and anabolic processes are temperature dependent so this simple reasoning may not hold, although a factor four in heat per biomass is a substantial change. This can be further investigated by also measuring gas exchange, especially carbon dioxide production, as has been done by Hansen et al. [49].

In the present results, we see an initial approximate proportionality between heat and mass of ergosterol, which later shifts to a continued increase of heat while the mass of ergosterol stays constant. What does this tell us about the fungi in these experiments? We discuss this qualitatively in terms of *growth* and *maintenance*—understanding that it is in practice impossible to assign, e.g., certain biochemical processes to either growth or maintenance as both naturally takes place simultaneously and rely on the same respiration process.

There is a fundamental difference between heat and ergosterol: heat is produced both by growth and maintenance, but ergosterol is only produced by growth. One can then interpret the present results as a first phase with growth followed by a second phase of maintenance.

During an exponential growth phase typical of microbial growth in liquid media both the rate of increase of biomass (growth) and the biomass itself (maintenance) are exponential functions. We start with a small inoculum containing only a negligible amount of ergosterol and a constant fraction of the biomass is ergosterol [1]; if there is a constant growth-related heat per new biomass and a constant maintenance heat production rate per old biomass; then measured heat per total mass of biomass (or ergosterol) will be constant. Even if fungal growth on a two-dimensional surface is not exactly exponential it is probable that a rather similar relation will hold. It is thus not unexpected that during a rapid-growth phase, ergosterol content is approximately proportional to produced heat.

Two different techniques were used in this investigation to quantify biological processes: isothermal calorimetry and ergosterol quantification. Some properties of these methods are given in Table 2 and it is seen that they are quite different in many respects. Such combinations of methods are needed to deal with the complexity of biological processes. Measurement of the unique fungal chemical marker ergosterol is an extremely specific, but destructive method that gives a result related to the biomass. It has, for example, been used to quantify fungal indoor contamination by measurements on house dust [31,43]. However, the correlation between biomass and ergosterol is complicated by that ergosterol is slowly degraded with time in dead hypha and that the formation of ergosterol may be dependent on species and environmental conditions. Isothermal calorimetry is a non-destructive laboratory technique that monitors the heat production of samples continuously and can thus be used to quantify the effect of, for example, toxic substances

Table 2
Overview of some characteristics of GC–MS/MS and isothermal calorimetry for fungal studies

Method name	GC–MS/MS	Isothermal calorimetry
Measures...	Ergosterol mass	Thermal power (heat)
Quantitative?	Yes	Yes
Fungal specific?	Yes	No
Destructive?	Yes	No
Measures only living fungi?	No	Yes
Expensive?	Yes	Yes
Primarily used for measuring...	Biomass (growth)	Respiration rate (growth and maintenance)
Continuous measurement?	No	Yes

on biological activity [50]. Calorimetry is an extremely general technique and cannot – for example, in a dust sample – differentiate between heat from moulds, house dust mites and condensation of water vapor. Calorimetry can only detect living fungi, whereas ergosterol determination is a way to assess previous fungal activity.

The use of the calorimetric technique gives us information on the growth rate of fungi on a two-dimensional substrate. Fig. 1 shows the thermal power for a representative sample from each group measured during long time. The same general behavior was found for all samples, indicating that the present type of calorimetric measurements can be used to test models of fungal growth [51,52] by measuring the thermal power produced by samples growing on, e.g., different substrates.

Future measurements could include not only the presently used techniques, but also measurements of biomass, nutrient concentration in the substrate, etc., to more fully understand the physiology of the fungi. Possibly, the combination of calorimetry and respirometry [29] could also indicate changes in used substrate.

4. Conclusions

We have measured heat and ergosterol production of a dry rot fungus and three mould fungi growing on agar. The ergosterol content of the growing fungal samples was initially approximately proportional to the metabolic heat produced, but later heat was still produced while ergosterol mass remained constant. The total amount of ergosterol produced was similar for all fungi, but the heat production differed significantly. For one mould, much more heat was produced per produced mass of ergosterol at 15 °C than at 20 °C, indicating that metabolic efficiency is temperature dependent. The combination of two very different methods of quantifying fungal activity is useful in studies of fungal growth.

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References

[1] S.Y. Newell, Methods Microbiol. 30 (2001) 357–372.
[2] J. Pietikainen, M. Pettersson, E. Bååth, FEMS Microbiol. Ecol. 52 (2005) 49–58.
[3] T. Yoshida, K. Uchida, H. Yamaguchi, Microbiol. Immunol. 41 (1997) 377–386.
[4] M.J. Carlile, S.C. Watkinson, G.W. Gooday, The Fungi, second ed., Academic Press, London, 2001.
[5] A. Ekblad, H. Wallander, T. Näsholm, N. Phytol. 1998 (1998) 143–149.
[6] A. Shapiro, J.T. Mullins, Mycologia 94 (2002) 267–272.
[7] J. Willcock, N. Magan, J. Stored Prod. Res. 37 (2001) 35–45.
[8] Y. Xie, J. Bjurman, L. Wadsö, Holzforschung 51 (1997) 201–206.
[9] M. Airaksinen, P. Pasanen, J. Kurnitski, Indoor Air 14 (2004) 55–65.
[10] K.F. Nielsen, G. Holm, L.P. Uttrup, P.A. Nielsen, Int. Biodeter. Biodegr. 54 (2004) 325–336.
[11] A.M. Harju, M. Venalainen, S. Anttonen, H. Viitanen, P. Kainulainen, P. Saranpaa, E. Vapaavuori, Trees (Berlin) 17 (2003) 263–268.
[12] L. Roberts, Isis 82 (1991) 198–222.
[13] A. Lavoisier, P.S. La Place, Mémoires de l'Académie des sciences 3 (1783) 355–408.
[14] R. Kemp (Ed.), From Macromolecules to Man, Handbook of Thermal Analysis and Calorimetry, vol. 4, first ed., Elsevier Science, 1999.
[15] H. Prat, Can. J. Bot.-Rev. Can. Bot. 30 (1952) 379–394.
[16] J.P. Belaich, in: A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, 1980, pp. 1–42.
[17] L. Gustafsson, C. Larsson, Thermochim. Acta 172 (1990) 95–104.
[18] L. Gustafsson, Thermochim. Acta 193 (1991) 145–171.
[19] L. Wadsö, F. Gomez, I. Sjöholm, P. Rocculi, Thermochim. Acta 422 (2004) 89–93.
[20] L.D. Hansen, B.N. Smith, R.S. Criddle, R.W. Breidenbach, J. Therm. Anal. Calorim. 51 (1998) 757–763.
[21] I. Lamprecht, R.S. Seymour, P. Schultze-Motel, Thermochim. Acta 309 (1998) 5–16.
[22] P. Beckman, Thermochim. Acta 205 (1992) 87–97.
[23] R.B. Kemp, Thermochim. Acta 193 (1991) 253–267.
[24] E. Schmolz, D. Hoffmeister, I. Lamprecht, Thermochim. Acta 382 (2002) 221–227.
[25] V.J.T. Van Ginneken, J. Vanderschoot, A.D.F. Addink, G.E.E.J.M. Van den Thillart, Thermochim. Acta 249 (1995) 143.
[26] J. Bjurman, L. Wadsö, Mycologia 92 (2000) 23–28.
[27] N. Markova, L. Wadsö, Int. Biodeter. Biodegr. 42 (1998) 25–28.
[28] L. Wadsö, J. Therm. Anal. 49 (1997) 1053–1060.
[29] L. Wadsö, Y. Li, J. Bjurman, Thermochim. Acta 422 (2004) 63–68.
[30] W.D. Grant, A.W. West, J. Microbiol. Methods 6 (1986) 47–53.
[31] L. Larsson, P.F. Larsson, Indoor Built Environ. 10 (2001) 232–237.
[32] J.D. Weete, Phytochemistry 12 (1973) 1843–1864.
[33] J.D. Weete, J.L. Laseter, Lipids 9 (1974) 575–581.
[34] L.M. Seitz, H.E. Mohr, R. Burroughs, D.B. Sauer, Cereal Chem. 54 (1977) 1207–1217.
[35] S.Y. Newell, in: G.C. Carroll, D.T. Wicklow (Eds.), The Fungal Community: Its Organization and Role in the Ecosystem, Marcel Dekker Inc., New York, 1992, pp. 521–561.

[36] G. Djajakirana, R.G. Joergensen, B. Meyer, Biol. Fert. Soils 22 (1996) 299–304.
[37] M. Klamer, E. Bååth, Soil Biol. Biochem. 36 (2004) 57–65.
[38] S. Marin, A.J. Ramos, V. Sanchis, Int. J. Food Microbiol. 99 (2005) 329–341.
[39] A.L. Pasanen, K. Yli-Pietila, P. Pasanen, P. Kalliokoski, J. Tarhanen, Appl. Environ. Microbiol. 65 (1999) 138–142.
[40] J. Bjurman, Int. Biodeter. Biodegr. 33 (1994) 355–368.
[41] A.W. West, W.D. Grant, G.P. Sparling, Soil Biol. Biochem. 19 (1987) 607–612.
[42] M.J.R. Nout, T.M.G. Bonants-Van Laarhoven, P. De Jongh, P.G. De Koster, Appl. Microbiol. Biotechnol. 26 (1987) 456–461.
[43] A. Sebastian, L. Larsson, Appl. Environ. Microbiol. 69 (2003) 3103–3109.
[44] M.H. Zwietering, I. Jongenburger, F.M. Rombouts, K. van't Riet, Appl. Environ. Microbiol. 56 (1990) 1875–1881.
[45] B. Gompertz, Philos. Trans. R. Soc. Lond. 115 (1825) 513–585.
[46] M.P. Levi, E.B. Cowling, Phytopathology 59 (1969) 460–468.
[47] J. Bjurman, H. Viitanen, Mater. Organismen (Berlin) 30 (1996) 259–277.
[48] L.M. Prescott, J.P. Harley, D.A. Klein, Microbiology, sixth ed., McGraw Hill, 2003.
[49] L.D. Hansen, C. Macfarlane, N. McKinnon, B.N. Smith, R.S. Criddle, Thermochim. Acta 422 (2004) 55–61.
[50] A.G.S. Prado, C. Airoldi, Anal. Bioanal. Chem. 376 (2003) 686–690.
[51] F.A. Davidson, J. Theor. Biol. 195 (1998) 281–292.
[52] F.A. Davidson, S. Olsson, J. Theor. Biol. 205 (2000) 73–84.