

PRINCIPLES OF A MICROCALORIMETRIC TECHNIQUE FOR THE STUDY OF MOULD ACTIVITY AS A FUNCTION OF RELATIVE HUMIDITY

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Abstract

Growth of mould fungi is one of the main causes of deterioration of foodstuffs, building materials and textiles. A new microcalorimetric technique has been developed for the study of mould activity as a function of water activity, temperature and atmospheric composition. This paper describes the method, in particular how to measure mould activity as a function of relative humidity.

Keywords: humidity, microcalorimetry, mould fungi, wood

Introduction

Mould fungi causes fouling and rejection of foodstuffs, building materials, textiles and other materials of organic origin. In contrast to other types of fungi, most mould fungi can grow at relative humidities much less than 100%. Spore germination at relative humidities as low as 64% has been reported [1], but for short term measurements most studies give the critical lower limit for growth at approximately 80% relative humidity of *Penicillium*, the most common type of mould [2-4].

Studies of fungal growth as a function of environmental parameters (relative humidity/water activity, temperature, atmospheric composition, *pH*) are normally carried out by measuring the growth of a large number of samples kept at different constant environment conditions. Growth is usually measured as radial growth, mass increase or as number of spores produced.

These different ways of assessing growth 'do not always show a close positive correlation' [2].

All metabolic activities produce heat. Therefore calorimetry may also be used as a measurement technique for the study of fungal activity. In the present paper is described a microcalorimetric method to monitor the thermal activity of mould fungi as a function of relative humidity (temperature and atmospheric composition may also be varied, but this is not discussed here).

In this paper relative humidity (ϕ) is used as a measure of the moisture state of both air and materials. For air

$$\phi = \frac{p}{p_{\text{sat}}}$$

Here p and p_{sat} are the vapor pressure and the saturation vapor pressure, respectively. For a material ϕ can be seen as the relative humidity of air in equilibrium with the material. It can, for the present purposes, be assumed to be numerically equal to the water activity (a_w) commonly used to express the moisture state of materials.

Method

In the technique described the thermal activity of a mould sample is measured in a microcalorimeter and the water activity of the sample is regulated in an external humidity generator.

The humidity generator is a fairly simple and inexpensive device that operates by mixing different proportions of air from two sources with different relative humidities. Figure 1 shows a schematic picture of the device. Room air flows through two 20 dm³ plastic boxes (A, C) and two 200 ml glass flasks (B, D) that have their bottoms covered with saturated aqueous salt solutions. The salt solutions will regulate the relative humidity of the air to approximately $\phi=0.54$ ($\text{Mg}(\text{NO}_3)_2$) in A and B and approximately 0.95 (KNO_3) in C and D, respectively. A timer controlled two-way valve (E) governs the relative humidity of the air stream by letting different proportions of air with these two relative humidities be mixed and continue to the sample.

The sample is contained in the same 3 ml calorimetric vessel (G) both in the microcalorimeter and in the humidity generator. This vessel can be connected to the humidity generator through two tubes without being opened. One of these tubes continues down to near the bottom of the vessel; the other ends on the bottom side of the lid. When in the microcalorimeter the tubes are sealed. The humidity generator flow path contains a millipore filter (F) to protect the sample from contamination. A peristaltic pump (J) draws air through the system which also contains a relative humidity sensor (I).

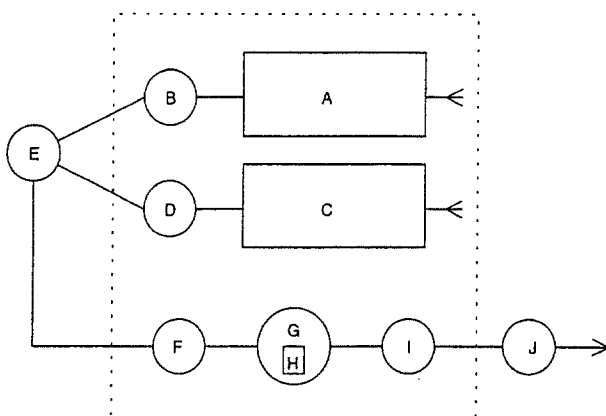


Fig. 1 Schematic picture of the humidity generator. The dotted line is the extent of the insulation. The components are discussed in the text

Except for the heat producing valve and pump, all components of the humidity generator are housed in an insulated box with a high heat capacity. The temperature in the box will not change more than a few tenths of a degree during the time (5 h) the humidity generator is used to achieve a new sample relative humidity.

As the temperature in A–D is the same as in G the relative humidity of the air entering the latter vessel (φ) may be calculated as a weighted mean between the relative humidities of the drier (φ_D) and the more humid (φ_H) air streams:

$$\varphi = \frac{\varphi_D t_D + \varphi_H t_H}{t_D + t_H} \quad (1)$$

Here, t_D and t_H are the times the valve is open to drier and more humid air, and φ_D and φ_H should be taken at the temperature in the humidity generator [5]. In the present instrumentation $t_D + t_H = 10$ s.

It is important that the moisture capacity of the flow path between the valve and the sample is high enough to even out the humidity differences between the drier and more humid air. In the present instrument the tubes of PVC had enough moisture capacity for doing this as no fluctuations were seen in the relative humidity measured by sensor I when the vessel G was removed from the flow path.

Discussion of method

The present technique is different from the most commonly used methods to assess mould growth.

1. It measures the thermal power (heat production rate) of the metabolic processes in the mould. This may not be proportional to other measures of mould growth, e.g. change in mass, as the metabolism of a fungi and corresponding enthalpy changes may change as environmental conditions change. Because of this I have here used the term 'mould activity' instead of 'mould growth'. Thermal power is of course the best measurable if one is interested in the energetics of the fungal growth (risk of self-ignition in bio-fuels, cooling needs in fermentor etc.).

2. The present technique monitors the activity of the fungi (except during changes of moisture state when the sample is in the humidity generator). Most other methods are used after a period of exposure to measure the sum of all activities during the exposure.

3. With the present technique it is possible to rapidly change the relative humidity of the sample during a measurement, thus making it possible to find how the thermal activity of one sample depends on its moisture state. It is therefore not necessary to use the large number of duplicate samples needed when each sample is exposed to only one constant climate.

4. Real hygroscopic substrates like wood, dried fruits and textiles may be used.

5. Only very small samples are needed. Typically, a 200 mg sample half-covered with visible mould is enough if the sample is not too poor in nutrients.

6. The microcalorimeter is very sensitive and can monitor the fungal metabolism as it takes place. It may therefore be possible to measure the mould activity

even under conditions of such low growth that it would take prohibitively long time to come to a result with conventional methods.

Figure 2 shows a schematic hypothetical measurement in which it is possible to see a number of interesting details:

- The measurement at A is continued until the thermal power is close to zero because of oxygen depletion in the vessel. From that type of measurement it is possible to gain information on how the mould fungus reacts to low oxygen levels (and high levels of carbon dioxide).

- Measurement G is made at the same relative humidity as measurement A, but the thermal powers are different. Such an anomaly may at least partly be caused by sorption hysteresis (an absorbing sample contains less moisture than a desorbing sample at the same relative humidity). Such measurements give interesting data on how air and sample humidity influences the activity of the mould.

- Measurements A and H are made at the same relative humidity and in the same mode of sorption (desorption). The observed difference in thermal power may be caused by the growth of the fungi during the measurement. A decrease in thermal power may be caused by nutritional depletion. It is advisable to start and end a measurement suite with the same relative humidity and mode of sorption to check whether the sample has changed during the measurement.

- In both measurements A and H the thermal power is not constant. It is possible with the present technique to study the dynamics of the mould activity, e.g. the rate at which a mould fungi can accommodate to a change in relative humidity.

- At relative humidity D the thermal power is very low. This indicates that the measurement is made at a relative humidity below the critical relative humidity. The present method is very well suited to the study of critical moisture levels for mould growth.

Figure 3 shows the result from a preliminary measurement with mould on a 300 mg wood sample. It is seen that the thermal power is a function of the relative humidity of the sample. The measurements at a relative humidity of 0.9 are continued until the thermal power drops because of oxygen depletion. At the other three

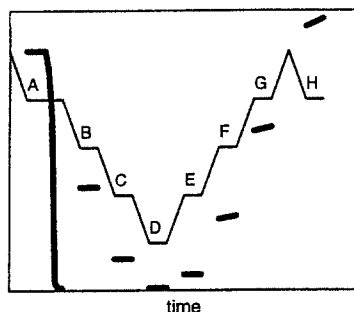


Fig. 2 Schematic hypothetical result from measurement with the present technique. The labelled parts of the measurement is discussed in the text. The thin line is the relative humidity of the sample and the thick line is the resulting thermal power

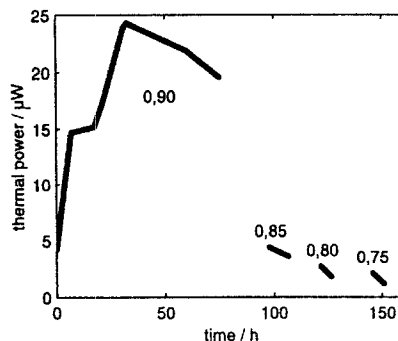


Fig. 3 Example of a measurement of mould growth on a 300 mg wood sample

humidity levels it is seen that the thermal power is decreasing; maybe because the mould is adjusting its metabolic rate to the new lower humidity levels.

Control of sample humidity

Changing the water activity of a sample can be made in a number of different ways:

- The sample can be placed in a container with constant relative humidity.
- Humidified gas can be flowed through a vessel containing the sample.
- In the case of absorption, liquid water can be added to the sample.

In the present work the second method was used.

Realistic studies of mould growth require that real substrates are used in measurements. Normal substrates, e.g. foodstuffs and building materials, are very hygroscopic materials. Changing the water activity of a hygroscopic sample requires that large amounts of humidified gas are flowed through the vessel containing the sample. Doing this down in a microcalorimeter during a reasonably short time will seriously disturb the thermal equilibrium of the calorimeter as high flow rates have to be used.

As an example, a wood sample of 200 mg at $\phi=0.70$ would require a flow rate of 5000 ml/h of air at $\phi=0.80$ to change the water activity of the sample to 0.795 in 2 h (using the method described below). Perfusion microcalorimeters are normally never used with higher flow rates than 100 ml/h, which would require 100 h of humidification. Therefore, an external humidity generator is used in the present work.

When humid air flows through a vessel with a sample the sample will absorb/desorb some moisture from the air if the sample and the air are not in equilibrium. Under perfect conditions all excess moisture will be sorbed. An example will illustrate this. If air with $\phi=0.80$ is flowed through a vessel with a sample with $\phi=0.88$, the air leaving the vessel will have a ϕ of 0.88 if the sorption process is perfect. Under less favourable conditions the relative humidity of the air leaving the vessel will be lower, e.g. $\phi=0.86$ ($\epsilon=0.75$, see below).

There may be several reasons for a sorption process to be less than perfect: 1. If the flow rate is high or the vessel is large, high boundary layer resistances and long vapor diffusion paths may limit the number of water molecules that can reach the sample (probably not a problem in our vessel). 2. Diffusional resistances within the sample (should not be a problem if thin samples, e.g. wood shavings, are used). 3. Time-dependent sorption ('non-Fickian behavior') may limit the rate of a sorption process (may be a problem for wood [6]).

A sample of mass M (g) at time t (s) has a moisture content u (g water per g dry sample) and (is in equilibrium with) a relative humidity of $\varphi(t)$. If this sample is exposed to an air stream with φ_a and a flow rate of F (m^3/s), the following mass balance equation is valid:

$$\frac{du}{dt} = \frac{\varepsilon F v_{\text{sat}}(\varphi_a - \varphi)}{M} \quad (2)$$

Here v_{sat} (g/m^3) is the saturation vapor content at the temperature of interest and ε is the efficiency of the sorption process (the ratio of the rate of sorption with the maximum rate of sorption).

Writing this equation only in terms of relative humidities gives:

$$\frac{d\varphi}{dt} = \frac{1}{\xi(\varphi)} \frac{\varepsilon F v_{\text{sat}}(\varphi_a - \varphi)}{M} \quad (3)$$

Here ξ is the moisture capacity of the substrate (the slope of the sorption isotherm, $\xi = du/d\varphi$). Rearranging and integrating both sides yields:

$$t_f = \frac{M}{\varepsilon F v_{\text{sat}}} \int_{\varphi_i}^{\varphi_f} \frac{\xi(\varphi)}{(\varphi_a - \varphi)} d\varphi \quad (4)$$

Here indices i and f indicate the initial and final states of the sorption process ($t_i=0$). This integral equation is numerically solved as ξ is a non-linear function of φ . Figure 4 gives an example of $\xi(\varphi)$ for wood.

If air with φ_a is passed through a vessel with a sample initially at φ_i , it will, in theory at least, take infinite time before the sample is in equilibrium with φ_a . In practice it is therefore necessary to accept a final value of the relative humidity of the sample (φ_f) which is close enough to that of the air (φ_a) to be acceptable (φ_f should not be set equal to φ_a in Eq. (4)). In the present work we have accepted a relative humidity of $\Delta\varphi=0.005$ below/above that of the relative humidity of the air, i.e.) $\Delta\varphi=\varphi_a-\varphi_f=\pm 0.005$ (+ for absorption and - for desorption).

Figures 5 and 6 shows the result from computer simulations to determine t_f for sorption on wood. Use the dimensionless time τ in the following equation to obtain t_f :

$$t_f = \tau \frac{M}{\varepsilon F v_{\text{sat}}} \quad (5)$$

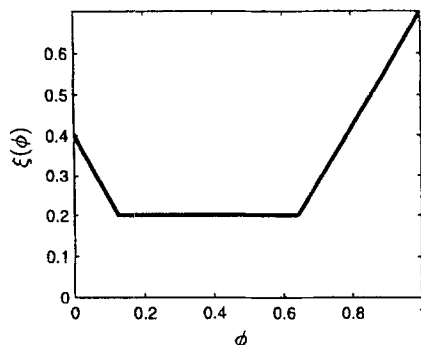


Fig. 4 Moisture capacity of wood used in the present simulations

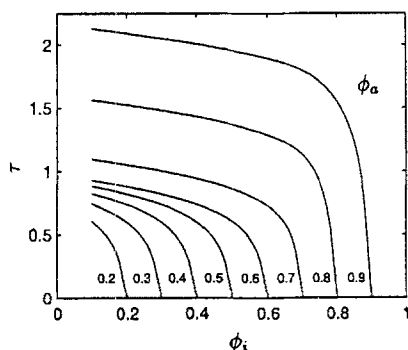


Fig. 5 Results from evaluation of Eq. (4) with absorption. Take ϕ_i from x -axis and curve corresponding to ϕ_a to find τ when $\phi_f = \phi_a - 0.005$

The example above with $\phi_i = 0.70$, $\phi_a = 0.80$ and $\Delta\phi = -0.005$ will give $\tau = 1.2$ from Fig. 5. Using $M = 0.2$ g, $F = 1.4 \cdot 10^{-6}$ m³ s⁻¹ and $\varepsilon = 1$ in Eq. (5) will give $t_f = 7500$ s at 25°C when $v_{\text{sat}} = 23$ g m⁻³.

In short relative humidity intervals where ξ can be considered constant, the following solution may be used:

$$\tau = \xi \ln \left| \frac{\phi_a - \phi_i}{\Delta\phi} \right|$$

In the present simulations $\xi(\phi)$ for wood was used (Fig. 4). As most hygroscopic materials have sorption isotherms of rather similar shape the results presented here may also be used for other materials provided a proper scaling is made.

The t_f calculated should be used with caution as it is a problem to know the efficiency ε of a certain combination of flow rate, material and vessel. In our measurement we have our sample in the humidity generator for 5 times the calculated t_f (calculated with $\varepsilon = 1$). Control measurements by weighing the vessel with the sample at each new humidity level and comparison with the sorption isotherm of the

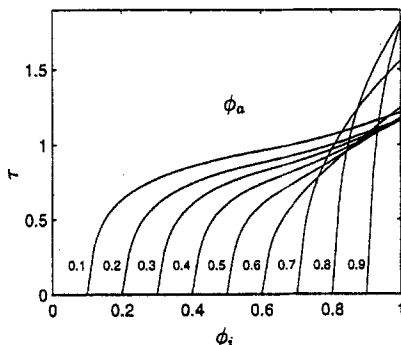


Fig. 6 Results from evaluation of Eq. (4) with desorption. Take ϕ_i from x-axis and curve corresponding to ϕ_a to find τ when $\phi_i = \phi_a + 0.005$

substrate is probably the only way to be confident in that the wanted moisture state has been obtained.

Conclusions

The presented technique to study mould activity as a function of relative humidity should be a valuable complement to the methods in use.

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