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Division of Building Materials

MOULD ON BUILDING MATERIALS

A calorimetric study of fungal activity as a function of environmental factors

Yujing Li

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Abstract

Mould problems in buildings have become a growing concern during the past decades. The growth of mould fungi indoors deteriorates air quality, influences human health and causes economical losses. Preventing mould growth from occurring is a more cost effective option than cleaning and renovation of buildings with mould problems.

Mould growth will occur in buildings when there are moisture problems. Many constructions have various defects that cause high humidity or even condensation on cold surfaces. Some of these defects are avoidable and can be corrected at the design stage. A calculation tool and knowledge of mould growth behaviour as a function of environmental parameters are therefore needed to predict the risk of mould growth for building design. The aim of this project was to study mould behaviour on building materials as a function of environmental parameters, such as temperature and relative humidity and therefore to serve as a tool for preventing mould problems in buildings.

Isothermal calorimetry is the main method used in this project to quantify mould activity. This method has not been applied much in fungal studies. Therefore it was tested and investigated to increase the understanding of the information that could be obtained from such measurements. Calorespirometry experiments (simultaneous calorimetry and respirometry) have been done on several mould fungi as well as on one rot fungus for understanding the correlation between fungal respiration and their heat production. Calorimetry was also compared with some traditional methods used in studying fungal growth by comparing heat produced by mould fungi with its biomass and ergosterol content. This also gave an increased understanding of fungal growth mechanisms. The results proved that calorimetry can not only be used on its own but can also be combined with other techniques to study fungal physiology.

The impact of temperature on mould growth was studied by comparing the produced heat, biomass and ergosterol of mould at five different temperature levels. Mould growth is highly influenced by temperature and this study also showed that the temperature at which that mould has the most rapid growth is not necessary the temperature at which it has its most efficient growth. This finding reveals the complexity of the influence of temperature on the fungal metabolism.

The influence of relative humidity on mould was also studied by measuring the fungal activities of mould growing on wood at different relative humidities. The results showed that although mould activities decreased when the relative humidity was low, too high relative humidity could also seem to inhibit part of its activity. Not only relative humidity but also moisture content is an important factor influencing mould activity. However the interpretation of these measurements was complicated by transient effects.

Mould growth development on wood, which were dried and treated at different temperatures was also studied by image analysis. All kiln-dried material exhibited higher mould growth levels than the air-dried material. Spruce heartwood had better resistance

against mould growth than spruce sapwood. Heat-treated spruce had very low levels of mould growth. The measurements confirmed that nutrient transport to the drying surface increases the risk of mould growth there.

Keywords

Calorimetry, isothermal calorimeter, mould, fungal activity, building, temperature, relative humidity, wood, image analysis, indoor environment

The papers included

Paper I **Measurements on two mould fungi with a calorespirometric method**
L. Wadsö, Y. Li, J. Bjurman, Thermochimica Acta 422 (2004) 63–68

Paper II **Correlating two methods of quantifying fungal activity: Heat production by isothermal calorimetry and ergosterol amount by gas chromatography–tandem mass spectrometry**
Y. Li, L. Wadsö, L. Larsson, J. Bjurman, Thermochimica Acta 458 (2007) 77–83

Paper III **An image analysis study of mould susceptibility of spruce and larch wood dried or heat-treated at different temperatures**
E. Frühwald, Y. Li, L. Wadsö, (*submitted*)

Paper IV **Produced heat, ergosterol content and biomass of a mould fungus as a function of temperatures**
Y. Li, L. Wadsö, L. Larsson, (*submitted*)

Paper V **A test of models for fungal growth based on metabolic heat rate measurements**
L. Wadsö, Y. Li, (*submitted*)

Paper VI **Measurements of fungal activity as a function of relative humidity by isothermal microcalorimetry**
Y. Li, L. Wadsö, J. Bjurman, (*manuscript*)

Preface

It is difficult to include everything one has done in research for five years into a few hundred pages' report. It is even more difficult to conclude what one experienced in five years within one page. These five years has become one of the most important parts of my life.

Before I started my PhD studies, I used to think a doctor must know everything. However, the more I learned the more I realized that there is so much more to learn. The more questions I tried to answer the more questions rose with each step of approach. I have been really confused but luckily I didn't give it up. Because there are so many reasons that I shouldn't.

I am very lucky to have Lars as my supervisor. He used to tell me that "There is no stupid question" and encouraged me to ask freely. I really appreciate that and I did ask a lot of questions, including many stupid ones. Thank you, Lars! From you I have learnt what a real scientist is. Thank you for all the help and trust. Without you I couldn't have come this far today.

I would also like to thank all the friendly colleagues in the Division of Building Materials. Although I didn't try my best in speaking Swedish but you have accepted me as who I am and helped me kindly, especially Marita and Britt for all the administrative work and Stefan and Bengt, who helped me enormously with my laboratory work.

A lot of thanks also go to all the PhD students who I got to know during my studies. Thanks for all the discussions, complaining and fun that we had together. It has been a great experience. And, Eva: thanks for the nice cooperation.

I also appreciate the great support from all the families and friends no matter far away in Asia or in Europe.

Last but not least, Frederic. I am not able to tell how much I appreciate all the love and support from you through these years. But above all, thank you for building a home for me, both in real and in heart.

Yujing
Lund, 30 July 2007

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THE BUMBLEBEE CANNOT FLY

"According to the latest theories of aerodynamics and as may be easily demonstrated by laboratory tests and wind tunnel experiments the bumblebee is unable to fly. This is because of the size, weight and shape of his body, in relation to the total wing spread makes flying impossible.

But … the bumblebee being ignorant of these profound scientific truths goes ahead and flies anyway – and manages to make little honey every day!"

Quote found in supervisor's office.

Chapter 1

Introduction

Mould growth in buildings has become a growing concern during the past decades (Becker 1984; Reijula and David 2004; Holloway 2006) and the situation does not seem to have improved. It has become an important environmental issue which has drawn a great deal of attention and resulted in a number of discussion and studies (Adan 1994; Dales et al. 1997; Nielsen 2001; Gorny et al. 2002). Mould growth is one factor that is suspected of deteriorating the indoor air quality (IAQ) of our buildings. The existence of indoor mould has been linked to negative impact on human health into large numbers of studies (Dales et al. 1994; Dales et al. 1997; King and Auger 2002; Kolstad et al. 2002; Bornehag et al. 2005; Chapman 2006). Mould also gives rise to discomfort among building inhabitants and leads to high remediation costs.

Mould growth and moisture problems are highly related in buildings since moisture is the key factor for mould growth indoors (Becker 1984; Adan 1994). Buildings with mould problems are common in warm and humid regions, such as East Asia during the summer monsoon season which brings constant raining for about one month. This is locally called "Plum Rain Season" (East Asian Rainy Season, the season when plums ripen, called "*Meiyu*" in Chinese or "*Baiu*" in Japanese) (Shimoda et al. 2005; EncyclopædiaBritannica 2007), which has the same pronunciation as "mould rain season" because mould easily grows in the buildings due to the warmth and the high humidity. However, mould problems are also common in countries with moderate or cold climate, such as in Northern Europe and Northern America (Nevalainen et al. 1991; Jones 1999; Norbäck et al. 2000; Wolkoff and Nielsen 2001; King and Auger 2002). It has for example been estimated that approximately 50% of all the buildings in Finland are moisture-damaged and prone to have mould problems (Reijula and David 2004). Although the outdoor vapour pressure is not very high in such regions, the indoor vapour pressure is always higher than that of the outdoor air because of the occupants and their indoor activities. This combined with lower temperatures outdoor than indoor during a large part of the year, can lead to condensation on interior walls, inside wall constructions, etc.

If one has problems with moulds in a building, removing moulds might temporarily improve the indoor air quality. However if the moisture problem remains the mould growth will most probably occur again after a certain period of time. Therefore the proper way to deal with

mould problems in the buildings is to reduce the moisture levels by renovating the building components where the mould problem occurs and by replacing the contaminated building materials, possibly with materials that are less susceptible to mould growth. Such renovation can take long time and the cost can be high. For example, a renovation project for a school with mould problems in Finland lasted for 5 years and cost over 5 million euros (Naaranoja and Uden 2007).

Preventing mould problems from occurring is a much more cost-effective way to deal with such potential problems than the remediation and renovation of buildings after an outbreak of mould growth. The prevention should be done from the building design stage. With proper tools, such as moisture calculation software, climate data, etc., it is possible to calculate the moisture state of building components. With a knowledge of how mould growth is influenced by environmental factors, it would be possible to predict the level of (or risk for) mould occurrence on building components. It should then be possible to improve the building design before the building is built to minimize or even prevent the mould problem from occurring. What we should have is the knowledge of mould growth behaviour on building materials under dynamic environmental conditions (temperature and relative humidity).

There are many studies of mould growth on agar medium, mostly made within the field of predictive microbiology in food science (Ayerst 1969; Sparringa et al. 2002; Dantigny et al. 2005; Pardo et al. 2005; Samapundo et al. 2005). However, as they are based on mould growth behaviour on agar nutrient media, which is very different from building materials, it is not clear if they can be applied in building science. However, there are a limited number of other studies of mould growth on wood and some other building materials (Viitanen and Ritschkoff 1991; Viitanen and Bjurman 1995; Nielsen et al. 2004; Johansson et al. 2005; Johansson 2006). The main limitation with these studies is that the methods of mould quantification used were mostly based on subjective grading of mould coverage into about five levels. Such results are difficult to use in mathematical modelling. Therefore there is a need for precise and quantitative method to study of mould growth on "real material", i.e. building materials, as a function of environmental parameters. Therefore this PhD project was initiated.

The ultimate aim of the work, of which this PhD-project is a part of, is to study how moulds behave on building materials in building environment as a function of physical parameters, e.g. temperature and relative humidity. Hopefully increased knowledge in this field can contribute to modelling of mould behaviour on building materials and further – combined with existing building physics calculation models – to predict the risk for mould growth.

The project was financed by three Swedish research foundations:

- FORMAS, Forskningsrådet för miljö, areella näringar och samhällsbyggande (*The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning*)
- KK- stiftelsen, Stiftelsen för kunskaps- och kompetensutveckling (*The Knowledge Foundation*)
- SBUF, Svenska Byggbranschens Utvecklingsfond, (*The Development Fund of the Swedish Construction Industry*)

Chapter 2

Indoor air quality

The importance of indoor air quality (IAQ) has been more and more emphasized during the last decades. The reason is simple: most people spend more than 90% of their lifetime indoors (Sarwar et al. 2003). The quality of the indoor air thus influences the health of all of us. One of the facts that has been noticed is that people who stay in a building with moisture problems (condensation on windows, water leaks, interior moulds, high relative humidity etc.) have increased risk of health problems (Bornehag et al. 2001). A general causal factor has not yet been identified, but it is probable that microbial growth plays an important role.

Modern houses provide people with thermal insulation, protection from the external environment and comfort for living. For example, the cooling and heating systems of modern houses will provide thermal comfort to the inhabitants. At the same time, the indoor environment is also relatively closed and is quite different from the outdoor environment. For example, the indoor air quality is largely influenced by the emissions from the building itself as well as the inhabitants' indoor activities. People can feel unwell or even ill by spending time in certain buildings. This is sometimes called the sick building syndrome (SBS) and is generally believed to be caused by bad IAQ (Lunau 1992; Jones 1999). The existence of high concentrations of indoor pollutants, including those of microbiological origin, leads to lower IAQ and increases the occurrence of SBS (Cooley et al. 1998).

2.1 Indoor air pollutants

The pollutants of the indoor air come from many different sources. They can be categorized as particle pollutants (mineral fibres, vegetable dust etc.); radioactive pollutants (most commonly radon); chemical pollutants (formaldehyde, carbon monoxide (CO), tobacco smoke etc.); biological pollutants (bacteria such as legionella, fungal spores etc.) (Samet and Spengler 1991; Leslie and Lunau 1992; Pluschke 2004). Normally these pollutants are airborne and can be inhaled into the human respiratory system, possibly entering our circulation system and therefore influencing our health.

Many particle (aerosol) pollutants come from outdoor environment, for example small (10 nm) combustion particles. They can also come from indoor sources, for example fibres peeled off from carpets and furniture; or by indoor activities such as cleaning. Such particles are normally in the order of a few μm and easily airborne.

The chemical contaminants, for example, volatile organic compounds (VOC), are emitted from building materials, furniture, adhesives, textiles, electronic devices, etc. (Jones 1999; Meininghaus et al. 2000). VOC also come from biological sources like humans, animals, microorganisms and plants in the building (Wolkoff and Nielsen 2001). For example moulds emit certain microbial volatile organic compounds (MVOC) that have a musty odour. The accumulation of carbon dioxide (CO_2) exhaled by the occupants is also a critical pollutant (although it is known not to influence health except at extremely high concentrations) (Appleby 1992). However, due to the more strict regulations on VOC emission levels, the focus of attention in the IAQ field has in the last decades moved from the problems of VOC and CO_2 to other subjects such as microbiological pollutants.

Microbiological indoor contamination includes mould and bacteria (and possibly other types of organisms), and substances produced by them. At least half of the air quality cases investigated by the National Institute for Occupational Safety and Healthy in the United States concern microbial contamination (King and Auger 2002). Among the microorganisms in buildings, moulds are considered to be the most interesting in relation to IAQ (King and Auger 2002).

Chapter 3

Fungal problems in buildings

Fungi, including moulds, i.e. filamentous fungi, and rot fungi are commonly found on humid building materials. The deteriorative, health and economical impact from such fungal growth indoors have been investigated in many studies. There has also been increased public awareness and concerns of indoor fungal problems, partly due to higher attention from the media. There have for example been many reports in American and European newspapers on mould problems (Armour 2002; Löfberg 2007; TT 2007).

Besides of the health problem triggered by the existence of indoor moulds, recent research have also showed that low IAQ can lower the productivity of the people working in such environments (Wargocki et al. 2000; Wyon 2004). Shortened working hours due to sick leave or lowered working productivity is an economical loss for employers. Due to health and economic considerations, buildings with mould problems must be cleaned and renovated. As mentioned earlier in Chap. 1, such renovation costs high both in time and economy. The importance of keeping buildings, not only homes, but also workplaces, free from fungi is obvious.

3.1 The fungi

The fungi are a biological kingdom of their own: Then Kingdom Fungi. They are different from plants, which have chlorophyll and thus have the capability of producing their own nutrients by photosynthesis (autotrophs); they are also different from animals, which have internal digestive systems and survive by ingesting and digesting other organisms as food. Fungi are heterotrophs but do not have internal digestive system: they survive by degrading other organisms and organic compounds to simple components and absorb them as nutrition for their own use by excreting enzymes to their surrounding environment (Carlile et al. 2001; Deacon 2001). Fungi clear away animal and plant debris and play an important role in the biological world as cleaners and enabling recycling of materials. Fungi exist everywhere and most often reproduce by large amount of spores. Moulds spores can resist harsh environment, such as dryness and low temperatures, and wait to germinate and grow until the environment is suitable (Carlile et al. 2001).

Although fungi are tough organism and can survive in harsh conditions, they rely on the moisture and nutrients in the ambient environment that diffuses or absorbed into the system

for their metabolism when growing. There are plenty of organic dusts, e.g. human skin scales, food particles, pollen and soil dust existing in our buildings. Building materials, such as wood materials, furniture and manufactured plasters, decorating finishes often contain organic compounds and additives (Adan 1994). The nutritional requirements for mould growth are minimal and therefore easily satisfied in buildings. Most of the fungi can also survive in a relative large temperature range, e.g. 0-50°C but their optimal growth temperatures are usually in the range 20-30°C, i.e. close to the normal indoor temperature. The indoor environment thus provides a nearly ideal environment for mould growth, except for the humidity level. Therefore when the humidity is elevated indoors, mould growth unavoidable happens.

Mould fungi and rot fungi are the two main groups of fungi found indoors when a building has moisture problems. Note that these groups are heterogeneous and are thus categorized like this for practical purposes only.

3.2 Indoor mould

Mould (also spelled "mold" in American English), refers to the conspicuous mass of mycelium (masses of vegetative filaments, hyphae) and fruiting structures produced by various fungi that appears on the surface of materials. Moulds are also called microfungi, as they do not have large fruit bodies (mushrooms) that macrofungi have (Gravesen et al. 1994).

Mould can be found on plants, soil and animal debris, i.e. almost everywhere in the outdoor environment. There is a similarity and connection between the common moulds found outdoors and indoors, as mould spores can be transported from outdoor to indoor (and vice versa) by air. However, there are also differences between outdoor and indoor moulds because different moulds have different adaptation to indoor and outdoor environments, e.g., temperature, humidity and material properties (Miller 1992). Some of the most common indoor moulds are species of the genera *Penicillium*, *Aspergillus* and *Cladosporium* (Adan 1994; Gots 2003; Schleibinger et al. 2004).

When growing indoors, moulds are mainly found on the surface of organic materials, such as foodstuffs, textiles, building materials etc. They can also grow on particles containing organic compounds, for example on house dust and on inorganic materials covered by dusts. By excreting enzymes, mould can to some extent decompose organic compounds, such as cellulose in wallpaper. However, they mainly live on low molecular mass substances and deteriorate materials mainly by fouling them. (Carlile et al. 2001).

Mould grows on substrate with high water activity (a_w) or high relative humidity (RH). They can be artificially categorised into three groups according to their minimum water activity requirement (a_{min}) for growth (Grant et al. 1989) (Fig. 3.1):

- Primary colonizers ($a_{min} < 0.8$)
- Secondary colonizers: ($0.8 < a_{min} < 0.9$)
- Tertiary colonizers ($a_{min} > 0.9$)

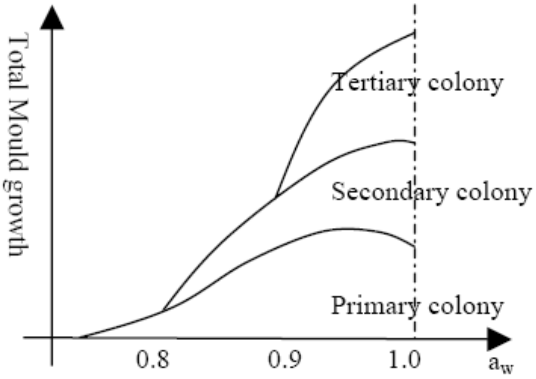

Figure 3.1 Schematic relationship between mould fungi colonizer groups and water activity.

Moulds included in the primary colonizers group do not demand high humidity for growth. They can also be called xerophilic mould (prefer dry conditions) as they can grow at relatively drier environment compare to the other two groups. The species in this group include *Penicillium, Aspergillus, Eurotiu, Wallermia*, etc. (Grant et al. 1989). They can be found on most materials in a house.

The secondary colonizer group includes moulds that demand a higher humidity environment to start growth. Their occurrence is normally in the places where the relative humidity is at high level, about 80-90%, and places with water condensation, such as bathrooms, kitchens and laundry rooms. Common species in this group include *Cladosporium, Alternaria, Phoma, Ulocladium*, etc. (Grant et al. 1989).

The tertiary colonizer group includes the mould species that only grow on substrates that have very high water content, i.e. where there is direct ingress of water that causes water damage to the substrate, rather than only humidity and "normal" condensation problems (Grant et al. 1989; Gravesen et al. 1994; Nielsen 2001). The species in this group include *Stachybotrys*, *Chaeotomium*, *Trichoderma* and *Auraeobasidium* and found for example behind leaking surface layers in bathrooms and on water damaged gypsum boards.

The normally accepted lowest a_w for mould to grow is 0.7-0.75 a_w (or 70%-75% RH) according to studies on agar media (Ayerst 1969). Grant observed that the minimum a_w for mould growth was 0.76 while Nielsen and co-workers found 0.78-0.80 as the minimum a_w level for most susceptible building materials (Grant et al. 1989; Nielsen et al. 2004).

If an organism can dry out without dying, i.e. it will start metabolising again when re-humidified, it is said to be desiccation tolerant. Moulds are generally desiccation tolerant in contrast to most animal and plants. Therefore mould normally starts growing as soon as the moisture level is suitable even after experiencing a long period of dry condition.

Another aspect of an organism's water relation is whether it can keep a constant high internal water activity in a drier environment. Organisms that can do this are called homoiohydric, in contrast to poikilohydric organisms whose internal water activity changes and more or less follows the ambient RH. Moulds are poikilohydric as they cannot control

the water activity in their hyphae because of their small size (this is also the reason why they are poikilothermic, i.e. that they do not have a constant body temperature.). Moulds are thus poikilohydric and desiccation tolerant, just as many terrestrial algae and lichens. Sometimes moulds are also said to be xerophilic (loving dryness) but this is a misleading term as the fact that they can survive dryness better than other organisms, does not mean that they love dryness.

It is also worth noting that there are relatively few organisms that live in our buildings compared to the outside environment, such as rot fungi, mites, spiders, a few insects, rats and mice. The main obstacle, if not including human's cleaning, is that most indoor environments are dry and there is a lack of light indoors. As mentioned earlier in this chapter, mould will most probably grow indoors where there are moisture problems.

Mould spores exist almost everywhere, from a few hundred to many thousands per cubic meter in normal indoor air. There is a seasonal variability in air spore content (normally higher in summer and lower in winter) (Gots 2003). Spores can survive for long time; some can even stay viable for more than 10 years, waiting for the suitable condition to germinate.

Mould produces large quantities of spores. For example, a colony arising from a single spore of *Penicillium* can produce millions or even up to 10^{12} of spores in a few days (Miller 1992). Most fungal spores are very small (2-20 μm) and therefore are easily airborne (Deacon 2001). Spore concentrations in the air can be high in a mould damaged buildings (although not at all as high as in some industrial settings such as sawmills and flour mills). Airborne spores can be inhaled into respiratory pathways. Every mould spore is a potential allergen due to its protein content. Inhalation of large amount of spores, which usually occurs in occupational settings rather than in homes or offices, can cause allergy and hypersensitivity pneumonitis (a syndrome caused by inhalation of high concentration of dusts containing organic matter including fungal spores) (Nicholls 1992). Exposure to moulds at both dwelling and non-industrial working environment has been linked to the symptoms of asthma, rhinitis and nasal symptoms and other illnesses (Kolstad et al. 2002; Reijula and David 2004).

Fungi can produce volatile organic compounds, so called microbial volatile organic compounds (MVOC) (Bjurman 1999) that can give an unpleasant smell in buildings with mould growth. MVOC can also be irritating when they come in contact with eyes, skins or the respiratory tract (Reijula and David 2004). Mycotoxin, secondary metabolites produced by mould fungi, are not volatile, but can be spread in the indoor environment when attached to airborne spores or mycelial fragments (Nielsen 2001). Mycotoxins can cause a toxic responds on higher vertebrates and animals, including humans, and some are potent carcinogent (Carlile et al. 2001).

Although the direct relation between mould growth in buildings and health in many ways is difficult to investigate and there are conflicting evidence published, it is generally accepted that the existence of mould in the buildings is unpleasant, and it can trigger a serie of health problems, such as SBS, allergies, etc. (Cooley et al. 1998; Bornehag et al. 2001; Bornehag et al. 2005).

3.3 Rot fungi in buildings

Although the main emphasis of this work is on mould fungi, the calorimetric techniques used in this project are quite general and can also be used for rot fungi (and many other types of biological samples). Some studies have therefore also been conducted with the dry rot fungus *Serpula lacrymans*.

There are several kinds of wood decay fungi found in buildings (Jennings and Bravery 1991). So called brown rot and white rot are predominantly basidiomycetes while soft rot fungi are often soil related fungi such as some ascomycota and deuteromycota (e.g. *Chaetomium*, *Fusarium* and *Paecilomyces*) (Carlile et al. 2001; Deacon 2001). The most serious one is the dry rot fungus *Serpula lacrymans*, the only rot fungus studied in this project. It has drawn much research attention not only because of its ability to attack timber, but also because it can spread through a building across non-nutritional surfaces and transport water through its strands (Nuss et al. 1991; Singh 1999).

Although the discussion on rot fungi in buildings is not at all as active as the discussion on moulds (at least in Sweden or in connection to IAQ), rot fungi are also important as they can cause large constructional damage in wood constructions (Bech-Andersen 1995). *Serpula lacrymans* is the most serious timber decay fungus in buildings in Northern and Central Europe, Japan and Australia. Destruction by rot fungi and removal of rot fungi in buildings can cause large economic losses (Singh 1999).

Chapter 4

Occurrence of mould problems in buildings

As mentioned in Chap. 3.1 mould growth will occur in buildings when there are moisture problems. Where and how the mould problems occur in the buildings will be discussed in this chapter.

4.1 Susceptible building materials

Fungal growth has been found on many different types of materials. Most common ones are organic materials such as wood and materials with organic components such as gypsum boards with paper surfaces (Hyvärinen et al. 2002). However mould growth can also be found on seemingly inert inorganic materials, probably because their surfaces absorb dust particles and in some cases contain substances like nitrogen that the fungi need (Hyvärinen et al. 2002).

4.1.1 Wood

Wood – usually from softwoods such as pine and spruce – is a common material in buildings: structural components, doors, windows frames, floors, ceilings, interior decoration, etc. Wood is natural organic material that has many good properties, such as high strength, natural origin and relative low price. However, its organic nature also makes it susceptible to fungal attack.

The cell wall of wood is composed by the three natural polymers cellulose, hemicellulose and lignin. In wood, several cellulose molecules are arranged parallel to each other, like the strand of a sewing thread. These are called microfibrils (Wadsö 1993). Microfibrils are coated with hemicellulose and embedded in lignin.

Cellulose is a polysaccharide composed only of glucose units. It is the main constituent of wood, making up 40-45% of its dry weight. The cellulose is partly crystalline and partly amorphous. Many fungi (including rot fungi and some mould fungi) produce cellulase that hydrolyses cellulose to cellobiose and glucose. Glucose can then be absorbed by the fungi as a carbon source (Prescott et al. 2003).

Hemicellulose is branched polysaccharide, or actually a group of polysaccharides, built from several different sugars, for example glucose, xylose, galactos, mannose, arabinose and 4-0-methylglucuronic acids of glucose and galactose (Viitanen 1996). Hemicellulose makes up about 25-30% of wood dry mass and can be degraded and used by many rot fungi (Carlile et al. 2001).

Lignin is an aromatic and amorphous polymer composed of phenylpropane units. The main role of lignin is to act as gluing material in the cell wall and to give sufficient rigidity to the cell wall (Viitanen 1996). The content of lignin in wood varies from 18% to 35% for different wood species. Lignin's aromatic nature also makes wood less attractive for degraders and most rot fungi cannot degrade the lignin except for only a few specialist fungi like the white rots.

The two most important nutrients needed by fungi are carbon and nitrogen. Nitrogen is the most important non-carbon nutrients as it is an essential part of amino acids, peptides and proteins. Wood is a rich carbon source for fungal growth, but it is poor in nitrogen. The carbon-nitrogen (C:N) ratio is in the order of 500:1, which is much lower than the requirement of about 30:1 for most fungi. However, rot fungi that use wood as nutrient sources are adapted to low-nitrogen substrates, for example by to some extent recycling nitrogen (Levi and Cowling 1969). Those fungi can grow inside of wood and degrade the wood cell walls. Other fungi (moulds) mainly grow on the surface of wood and do not degrade the wood cell walls as they lack strands which can penetrate wood pores or have no enzymes which can degrade wood cells as rot fungi. They live on soluble sugars and other compounds from the wood itself or from dust, etc. absorbed on the surface.

Wood used in buildings is dried at elevated temperature in order to reduce its water content before use. However, the drying process transports the nutrients together with moisture to the surface of the wood. When the water evaporates to the air, the nutrients are left on the surface of the wood (Theander et al. 1993). These nutrients are then become available for the mould fungi that cannot penetrate into the wood material.

Wood for external applications is often treated with copper or copper-chromium-based preservatives against rot fungi. Such treatment is seldom used for wood inside of the building and it does not prevent mould growth. Other preservatives like boron compounds and tertiary amine compounds can be used to decrease mould growth, but the use of these is rather limited for environmental reasons.

Besides wood, wood-based board materials are also used extensively in modern constructions. They are usually divided into the following groups:

1. Fibre-boards of different densities made by pressing together wood fibres under high temperature and pressure (no added adhesive).
2. Particle boards in which wood particles are held together with an adhesive.
3. Medium-density fibre-board (MDF) in which fine wood particles are held together by an adhesive.
4. Plywood in which veneers are held together by an adhesive.

Except for the rare cases in which wood-based boards are treated with fungicides, their resistance to attack by fungi is similar to that of wood (Wang 1994).

4.1.2 Paper

Paper covers such as gypsum plaster board and wall paper are also susceptible to fungal growth as they are made from wood. Furthermore, there might be other organic material added – such as the glue that attaches wall paper to the wall – that can also be utilized by fungi.

4.1.3 Polymers

Polymeric materials can also be susceptible to mould growth if there are components of the polymers that the fungi can use as nutrients. One such reported case concerns ester-based plasticizers in polyvinyl chloride (PVC) (Webb et al. 2000). But there can also be mould growth when there are organic fragments, such as dust accumulated on the surface.

4.1.4 Inorganic materials

Fungal growth can also be found on inorganic materials, such as concrete and brick-work (Webb et al. 2000). The reasons can be various. There might be dust or some other particles containing organic fragments accumulated on the surface of such materials which provide nutrition for mould growth. There might also be other nutrients, such as nitrogen, in the materials, which provide extra trigger for mould growth.

4.2 Indoor temperature

Buildings in colder climate demand good insulation in order to reduce the heat loss. Due to comfort and energy saving considerations, the ventilations of the buildings are normally controlled in order to reduce the heat loss through the ventilation. The indoor temperature is normally kept at around 20°C, and there should be no draft, no cold surfaces etc. in order to fulfil the comfort of the building occupants and their activities (Lunau 1992).

To fulfil the heat insulation and structure purposes, modern buildings are built using rather complex constructions containing many different materials in different layers, for example an outer wall can be made from bricks, two types of boards, mineral wool, wood, a plastic foil and a gypsum board. This is different from traditional buildings that were built with fewer materials in homogeneous constructions, for example massive brick or wood walls.

The structures of the buildings are complex. There are unavoidable compartments of the building where the surface temperature is hard to keep at a similar level as the room temperature due to different kinds of heat loss through thermal bridges, for example the cold corners where the walls are connected, around windows and doors, the connection between ventilation ducts and the walls etc. The temperature at such parts will be lower than the average surface temperature. It can drop down to 10°C or even lower especially during the cold seasons (Burke et al. 2002).

In some parts of the building the surface temperature can be increased during the summer season. For example some building roof can be heated up by strong solar radiation and the temperature of the attic surface can reach as high as over 45°C.

The temperature in most parts of a building are within the 0-50°C range in which mould fungi can grow most of the year. Temperature is therefore not a factor that determines if one gets mould growth or not; it only determines the rate of mould growth.

4.3 Moisture in buildings

The vapour pressure and vapour content (gram water vapour per cubic meter of air) is always higher indoors than outdoors as people, plants and activities such as cooking and washing produce moisture. It is normally considered that indoor sources adds about 4 g/m^3 (Nevander and Elmarsson 1994) extra moisture content to the indoor air, but this can vary greatly depending on the ventilation and the activities in a building. The ventilation is needed to remove this excess moisture and prevent the water vapour content from becoming too high. Higher ventilation rate will reduce the excess vapour content.

4.3.1 Vapour content, temperature and relative humidity

As mentioned in Chap. 4.2 the temperature indoors can be uneven due to heat losses through thermal bridges. The relative humidity on a cold surface or a cold corner will increase due to the physical properties of water vapour as described in a psychrometric chart (Fig. 4.1). For example, if the relative humidity (RH) of the room air is 50% when it is 23°C, the water vapour content is about 10 g/m^3 (a normal level indoors). A vapour content of 10 g/m^3 will have a RH of 75% at 15°C and close to 100% (dew point) at 10°C. Therefore the temperature drop will increase the relative humidity. As moulds grow when the RH is above 75% they will in the above case grow where an interior wall has a temperature of 15°C or lower.

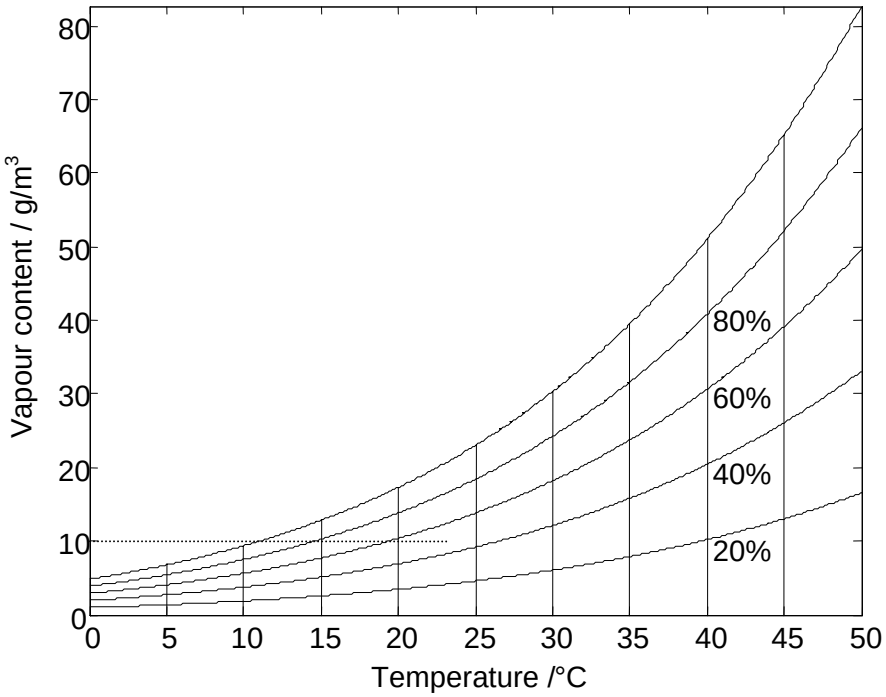

Figure 4.1 A simplified psychrometric chart.

4.3.2 Leakage of liquid water

A second reason for high relative humidities – the first one is being cooled humid indoor air – is leakage. This can be from water pipes or leakage of rain water through cracks and other building defects, or places like bathrooms where liquid water is used in a building and can

penetrate the inner surface and penetrate into the wall structures (Jansson 2006). Liquid water ingress can quickly lead to high relative humidities supporting growth of rot fungi or moulds.

4.3.3 Moisture, the key factor

As mentioned in Chap. 3.1 moulds grow indoors needs three factors: nutrition, temperature and moisture. Temperature is nearly always within the range acceptable for moulds and there are many organic materials available indoors, e.g. wood, paper and dust. Nutrition and temperature are thus not factors determining if moulds grow indoors. The relative humidity level in buildings can vary from as low as 10% to as high as 100% at different times and places. Therefore moisture is the key factor for mould growth indoors (Fig. 4.2).

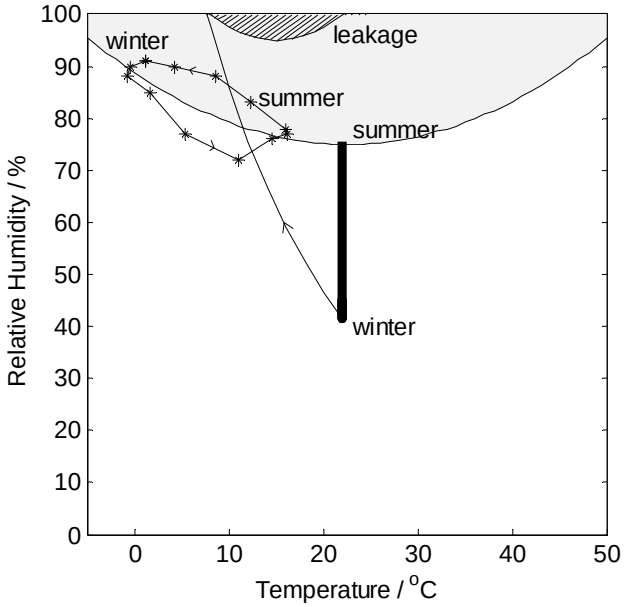

Figure 4.2 Schematic description of the moisture conditions in a building in a temperate climate. The gray area shows (very schematically) the RH-T-area where there is risk of mould growth. The cycle with stars shows how RH(T) outdoors changes throughout a year. The thick line is RH(T) indoors assuming a 4 g/m^3 vapour excess and that the temperature is constantly 22°C. The thin line shows how the RH increases if the temperature of the indoor air decreases in wintertime. The dashed area shows moisture states resulting from leakage of liquid water. The data are monthly mean values for Sturup in the south of Sweden. Note that, e.g. humid and warm years can be significantly different from the mean values shown in the figure.

4.3.4 Moisture problems indoors

There are different reasons why one can get high humidity levels and mould growth in buildings. First of all there are some building components that simply cannot be held constantly at enough dry condition to completely avoid mould growth, for example external wood panelling (cf. Fig. 4.2). Secondly buildings are sometimes designed in such a way that we will get mould problems. A recent example is that it is still common to build ventilated crawl space containing wood, a construction in which one often find substantial mould growth. Thirdly, high humidities and mould growth are often the result of defects in buildings. For example leakage of humid room air to a cold attic through a leaking hatch. Here are some typical examples of building parts where moisture problems can occur:

- **Attic**
  In the winter-time un-insulated attics are cold, especially the wooden claddings often used under the roofing material. If air from the floor below is allowed to leak into the attic, condensation can occur. Many such cases have occurred in Sweden, especially after insulation has been added on the attic, making it colder. If the ventilation of the attic (natural ventilation with outdoor air) is low, massive mould growth can occur in a short time. This will not happen if the construction is air tight between the attic and the floor below.

- **Crawl-space**
  A crawl-space is a kind of foundation separating a building from the ground. The height of crawl-space is about 1 m and there is normally no insulation between the crawl-space and ground. In most cases the crawl-space is ventilated with outdoor air. In wintertime the crawl-space temperature decreases and when the spring comes with warm and humid air water vapour may condense in the still cool crawl-space. This is a problem in a large fraction of Swedish buildings with such craw-spaces.

  There are certain types of crawl-spaces that do not get these problems, for example "warm crawl-spaces" which have thicker insulation between the ground and the crawl space (Matilainen et al. 2003). Slab on the ground construction with the insulation under the slab is also a much safer construction than ventilated crawl-space.

- **Insulation material behind rendering**
  Today a common way to build houses in Sweden is a construction with rendering on thermal insulation. The actual load carrying frame can consist of wood. The rendering is often only a few millimetres thick and is applied directly on the thermal insulation. Because these walls are non-ventilated and non-drained, they are very sensitive to incoming water. When there is a water-leak the water has nowhere to go when inside of the construction and this can lead to moisture problems. One cannot allow any leakage of water into such constructions, but this can be very difficult to avoid in practice, especially with connections between the façade and window frames, balconies and so on. There has been observed many cases of incoming water through leakage with this type of construction, which makes them potential places for mould growth (Jansson et al. 2007).

- **Ventilation systems**
  As large volume of air is drawn through ventilation systems the particle in the air can be deposited in the systems. Mostly this takes place in the filters but also on other components. If filters are not changed regularly or if, e.g. the ducts are never cleaned there can be mould growth in the deposited dust. Emissions from this mould growth are transported into the indoor environment.

- **On surfaces in bathrooms**
  In bathrooms the humidity is high every time someone takes a shower or a bath, and it is often seen that moulds grow on tiles, PVC covers, shower curtains etc. Regular

cleaning and ventilation will decrease this problem since that is quite unavoidable if one does not use fungicide treated materials.

- **Behind of the tile in bathrooms**
  The most common system to build tiled bathrooms in Sweden consists of gypsum boards, impermeable layers and tiles. It has been reported that many such constructions show heavy mould growth (Jansson 2006) on the gypsum board as the "impermeable layer" and the tile joints are permeable to water. It would, from the moisture design point of view, be better to use welded PVC coatings or constructions based on inorganic material for such building components.

- **Water leakage**
  A trivial source of moisture in buildings is leakages from pipes or condensation on cold pipes. This often brings high moisture content and growth of rot fungi, but it may also support mould growth.

Chapter 5

Method - calorimetry

The aim of the project was to study fungal activity as a function of different environmental parameters. The fungal activity is reflected by the heat produced by the fungal metabolism. The measurement of heat and heat production rate is called calorimetry. In this project isothermal heat conduction calorimetry was used to measure the thermal power produced by the fungal samples. It measures heat production rate (thermal power) from samples at constant temperature. We take the thermal power as a measure of the activity of the fungi at a certain specific constant condition. The general idea was to make calorimetric measurements with different environmental parameters, thus mapping the activity of the fungi as function of the environmental parameters (most importantly water activity and temperature).

5.1 Calorimetry in biological studies

Nearly all processes (chemical, physical and biological) produce heat. Heat is a form of energy that always accompanies life processes. The reactions by which organic carbon sources are broken down are used to obtain energy to support life. All biological processes are accompanied by production of heat and all metabolisms are exothermic, i.e. produces heat, and this heat is continuously released to the surroundings. In a system with constant pressure (as is the case for biological systems) the release of heat can be directly related to the change in enthalpy (ΔH).

According to the first law of thermodynamics, the conservation of energy, energy can neither be created nor be destroyed. It only can be transferred and converted between different types of energy. The lowest form of energy is heat. All other types of energy - chemical energy, potential energy, etc. - are called work and can be completely converted to heat. Heterotrophic organisms get their energy from the chemical energy of the substrates they metabolize. When they have utilized the substrate to make new biomass, substances with lower energy content (for example carbon dioxide) and heat remains.

Most organisms rely on aerobic metabolism (respiration) to obtain energy for sustaining activities and life. Respiration is essentially a combustion producing water and carbon dioxide, as was proved by Lavoisier and LaPlace by calorimetric experiments in 1780

(Lavoisier and La Place 1783). The enthalpy change during the combustion of most organic substances is directly proportional to the number of atoms of oxygen consumed during combustion ($\Delta H \approx$-465 kJ/mol O_2). This is called "Thornton's law" (Thornton 1917; Battley 1987; Battley 1999) and is valid both for biological and chemical processes.

The most basic aerobic metabolism is glucose degradation (Battley 1999):

$$\text{glucose} + 6O_2 \rightarrow 6H_2O + 6CO_2 \qquad \Delta H = -469 \text{ kJ/mol } O_2 \qquad (5.1)$$

For every mol of oxygen consumed for this aerobic reaction, about 469 kJ heat is produced (obeying Thornton's law). The enthalpy value is (given as heat per mol oxygen) relatively the same for other substrates besides of glucose (the exact value of the enthalpy also depends on other factors such as whether the produced carbon dioxide ends up dissolved in water or in the gas phase). All organisms (humans, mammals, invertebrates, fungi etc.) follow the above equation when they are degrading substrate to carbon dioxide in aerobic metabolism. This rule therefore establishes a general connection between respiration and heat production. The heat production rate is proportional to metabolic rates counted per mole oxygen consumed and provides a direct indication of metabolic responses such as reaction to stresses.

Thornton's rule is valid only for heat per amount consumed oxygen. As respiratory quotients (mol carbon dioxide produced per mol oxygen consumed) depend on substrate (Gnaiger and Kemp 1990), heats per mol carbon dioxide consumed will not be the same for all aerobic processes, but depend on the substrate.

Calorimeters have been applied in the study of different biological systems (references given below are a few examples).

- Applied studies in biochemical engineering (Duboc et al. 1999)
- Fundamental studies of yeast metabolism (Larsson and Gustafsson 1999)
- Aquatic animals (Normant et al. 2004)
- Insects (Lamprecht and Schmolz 1999; Schmolz and Lamprecht 2000)
- Animal physiology and bioenergetics (Hand 1999)
- Animal tissues (Kemp and Guan 1999)
- Human cells (Monti 1999)
- Plant metabolism (Criddle et al. 1988; Criddle et al. 1991; Hansen et al. 1995; Criddle and Hansen 1999)
- Vegetable respiration (Gómez et al. 2004; Wadsö et al. 2004)
- Microbial degradation of foodstuff (Shiraldi et al. 1999; Alklint et al. 2005)

There are only a few calorimetric studies on fungi in the literature (not counting studies on yeast as mentioned above): (Wadsö and Bjurman 1996; Wadsö 1997; Xie et al. 1997; Wadsö et al. 2004; Li et al. 2007).

5.2 Theory of isothermal calorimetry

The isothermal calorimeter (often called conduction or heat conduction calorimeter) works by conducting the heat produced in a sample away from the sample through a heat flow sensor to a heat sink. The heat flow sensor is normally a thermopile consisting of about 100

semi-conductor thermocouples. An identical calorimeter with an inert sample is used as a reference to reduce noise (twin calorimeter). The reference is normally water with the same heat capacity as the sample. The calorimeter is placed in a thermostat to keep it at constant temperature. Fig. 5.1 shows a schematic drawing of an isothermal calorimeter and a typical output from a measurement.

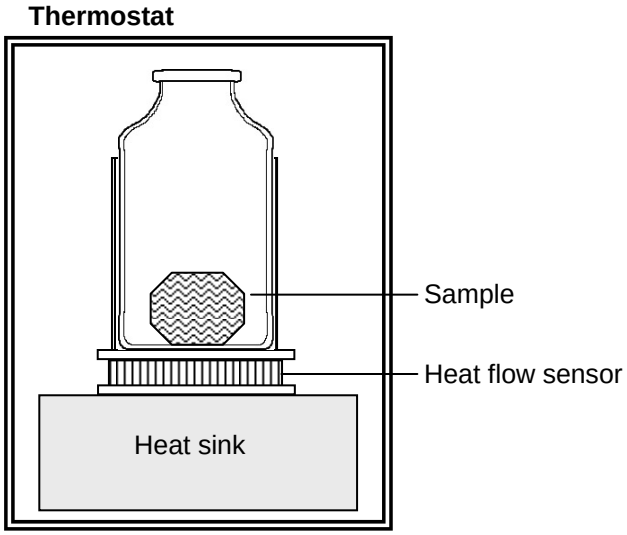

Figure 5.1 Schematic principle of an isothermal calorimeter.

As heat is produced by the sample, the sample gets slightly warmer than the heat sink, and heat then flows through the heat flow sensor. The heat flow sensor generates a differential voltage, which is proportional to the temperature difference over the heat flow sensor. This differential voltage is then amplified and measured by a data logger connected to a computer. The signals (in volts) are converted to thermal power values (in watts) by multiplication with a calibration coefficient (in watts per volts) that is obtained by electrical calibration.

Note that the term "isothermal calorimeter" does not imply that the measurement takes place under perfect (thermodynamically) isothermal conditions. There will be small temperature changes in the sample, but as long as these are small enough to give the same result as had the measurement been perfectly isothermal, it can be called isothermal calorimetry.

There are two types of isothermal calorimeters used in this project:

- The TAM isothermal microcalorimeter (Thermometric, Järfälla, Sweden). With a liquid thermostat with a 24 h temperature stability of 0.1 mK, this calorimeter is very sensitive. Its accuracy is lower than 1 μW. There are four twin calorimeters in the TAM used. The sample is placed in 1-4 ml vials of glass or stainless steel. Our calorimeters and the electronics were partly home-built, but by similar in design as a TAM.

- The TAM Air isothermal calorimeter (Thermometric AB, Järfälla, Sweden). This isothermal heat conduction instrument contains eight twin calorimeters placed in an air thermostat with a 24 h temperature stability of 0.02 K. The samples are places in 20 ml glass vials that are sealed with Teflon coated rubber seals and aluminium caps. This calorimeter is less sensitive than the TAM microcalorimeter. Its accuracy is about 10 μW, which is enough for many practical applications, e.g., in biology. This instrument has the advantage of low cost per calorimetric channel and very easy operation.

Chapter 6

Fungal studies by calorimetry

The major part of this thesis concerns the use of isothermal calorimetry in the study of fungi. Here follows a summary of the calorimetry related papers, with some new material added.

6.1 Heat production and respiration of fungi – calorespirometry

Paper I concerns calorespirometry, the combination of calorimetry (measurements of heat) and respirometry (measurements of gas exchange, i.e., rate of oxygen uptake and/or carbon dioxide production). The most obvious connection between calorimetry and respirometry is – as mentioned above – that the complete combustion of almost any organic compound produced a heat of about 465 kJ per mol oxygen consumed (Thornton 1917). This together with the assumption that the respiratory quotient (RQ, mol CO_2 produced per mol O_2 consumed) is 1.0 is the basis of indirect calorimetry in which one measures carbon dioxide production and calculates heat production. However, the model is only valid for steady-state systems, i.e., systems that are mature and not changing in any way. Hansen and co-workers believe that this is seldom the case and that most applications of indirect calorimetry actually have been made on non-steady state systems and therefore more or less inaccurate (2004). Calorespirometry, the simultaneous measurement of calorimetry and respirometry, can give more accurate information on for both steady-state and non-steady-state systems on their heat production and respiration behaviours.

6.1.1 Calorespirometer

A rapid calorespirometric method to determine the effect of decreasing O_2 and increasing CO_2 pressures on fungi and other organisms was developed (paper I) based on the principles used by Hansen and co-workers (Criddle et al. 1988; Criddle et al. 1991; Hansen et al. 2004). The calorespirometric device consists of two isothermal calorimeters (Fig. 6.1) that are placed in a TAM Air thermostat (Thermometric AB, Järfälla, Sweden). The calorimeters are modified TAM Air calorimetric units. Two 20 ml glass vials are placed in the calorimeters and are connected by a tube that passes through a slit that has been cut through both calorimeters. The sample is placed in one of the vials and a CO_2 absorbent is placed in the other vial. Aqueous NaOH (0.4 M) (Criddle et al. 1990; Criddle et al. 1991) was used as CO_2 absorbent. A valve is positioned on the tube and is controlled by a computer-controlled step

motor placed outside the thermostat. Each vial is connected to an external pressure sensor through a thin tube.

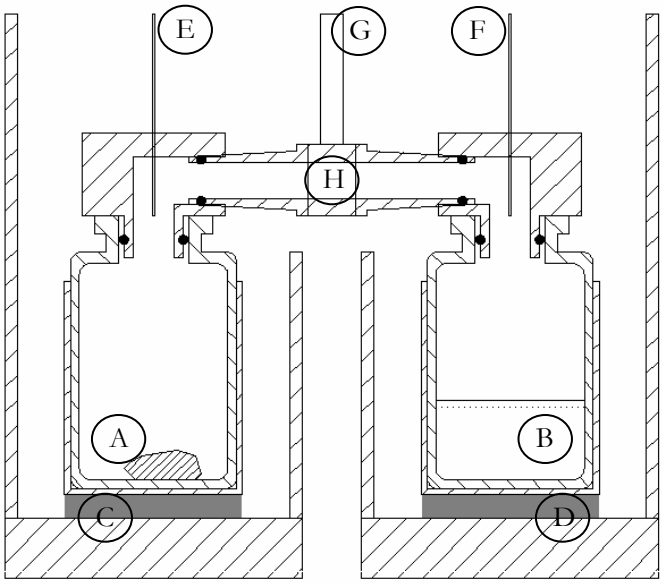

Figure 6.1 A schematic drawing of the calorespirometric device. A: Sample; B: CO_2 absorbent solution; C: sample calorimeter heat flow sensor; D: absorbent solution calorimeter heat flow sensor; E: sample vial pressure sensor tube; F: absorbent vial pressure sensor tube; G: shaft to turn valve; H: valve. Note that both the shown calorimeters are twin calorimeters with references (behind the shown calorimeters).

6.1.2 Material and method

Two mould fungi and one rot fungus were studied with this calorespirometer:

- *Penicillium roqueforti*; strain from Biocentrum, Technical University of Denmark.
- *Penicillium camemberti*; strain from Biocentrum, Technical University of Denmark.
- *Serpula lacrymans*; strain from Haavard Kauserud, Oslo University, Norway (not in paper I).

For a measurement, the biological sample was placed in the sample vial and the CO_2 absorbent was placed in the absorbent vial (Fig. 6.1). The vials were then placed in the calorimeters and the valve was left open for 1 h. Afterwards the valve was switched at certain interval. The thermal powers of sample and absorbent, and the pressures in each vial were recorded continuously. The disturbance from switching was small and could be neglected. The measurement started at aerobic conditions (20.9% O_2; corresponding to 21.3 kPa at an air pressure of 101.5 kPa) and continued into the anoxic phase when the O_2 was consumed. It was then possible to flush the device again with air and repeat the measurement to check if the sample had grown or suffered damage during the first measurement.

The amount of oxygen consumed and carbon dioxide produced can be calculated from both the pressure change and the heat production by absorbent (the enthalpy of reaction between CO_2 and 0.4 aqueous NaOH is 108.5 kJ/mol (Criddle et al. 1991)). A gas exchange ratio

(mol oxygen consumed per mol carbon dioxide produced) and the enthalpy of metabolic processes (heat produced per mol of CO_2 produced) were also calculated.

6.1.3 Results and discussion

The primary results of the measurements on the two mould fungi *P. roqueforti* and *P. camemberti* are shown in Figs 6.2a-b and 6.3a-b. The evaluated parameters are shown in Figs. 6.2c-e and 6.3c-e.

The thermal power of *P. roqueforti* (Fig. 6.2) decreased slowly when the O_2 pressure decreased from 21.3 kPa to about 1 kPa. At lower O_2 pressures the thermal power decreased rapidly to low values. The metabolic gas exchange rate was about 1.0 when O_2 pressure was above about 1 kPa and then decreased to about zero. The metabolic enthalpy of *P. roqueforti* was about 470 kJ/mol(CO_2) under aerobic conditions and about 30 kJ/mol(CO_2) for anaerobic conditions.

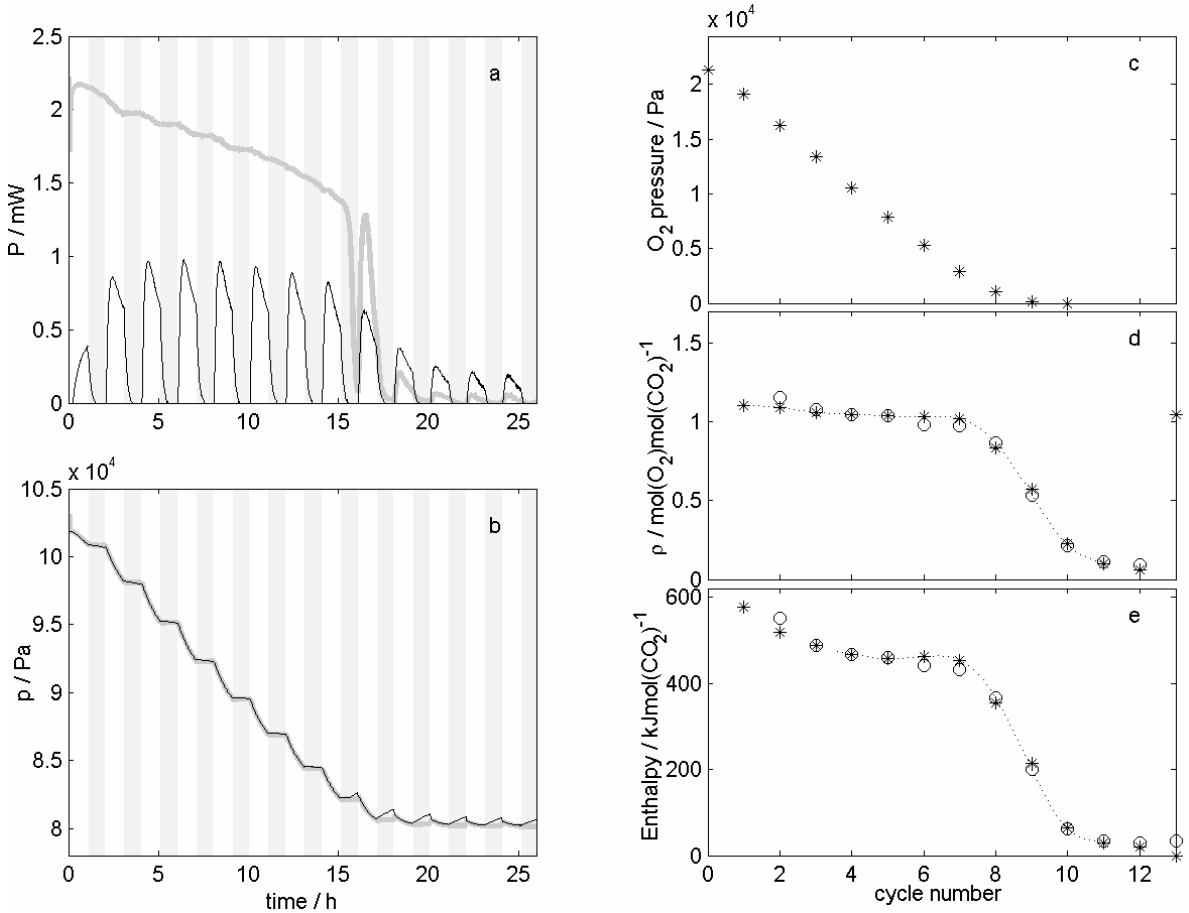

Figure 6.2 Result of measurement with *P. roqueforti* growing on malt extract agar. The gray fields show when the valve is closed. a. Thermal power from *P. roqueforti* (gray line) and CO_2 absorbent (black line); b. Pressure changes measured in sample vial (gray line) and CO_2 absorbent (black line); c. O_2 pressure; d. Metabolic gas exchange ratio; e. Metabolic enthalpy change.

The results of measurements on *P. camemberti* (Fig. 6.3) were similar to those for *P. roqueforti* given above, except that the O_2 threshold was higher (the thermal power dropped to low values at 2-3 kPa). This suggests that *P. roqueforti* can stand lower O_2 concentrations than *P. camemberti*, which agrees with the known physiology of these two species (Samson et al. 2000).

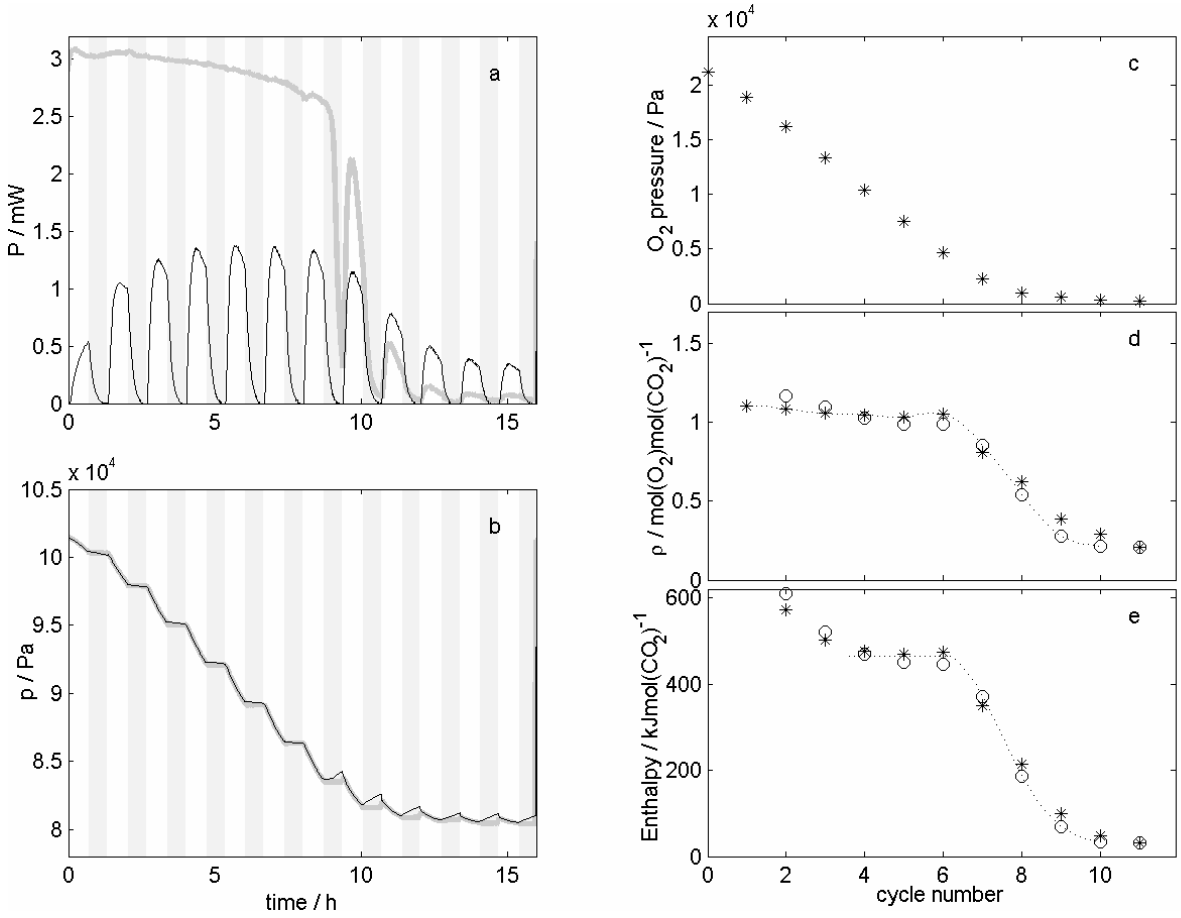

Figure 6.3 Result of measurement with *P. camemberti* growing on malt extract agar. The gray fields show when the valve is closed. a. Thermal power from *P. camemberti* (gray line) and CO_2 absorbent (black line); b. Pressure changes measured in sample vial (gray line) and CO_2 absorbent (black line); c. O_2 pressure; d. Metabolic gas exchange ratio; e. Metabolic enthalpy change.

The measurement on *S. lacrymans* (not given in paper I) is shown in Fig. 6.4 and was done by the same calorespirometer as used for measuring mould fungi, except that the valve was controlled manually. The thermal power of *S. lacrymans* decreased slowly before oxygen pressure dropped below about 3 kPa. The rate of oxygen consumption and carbon dioxide production rate were relatively constant above this oxygen pressure level indicating that the rot fungi can grow under conditions of reduced oxygen levels.

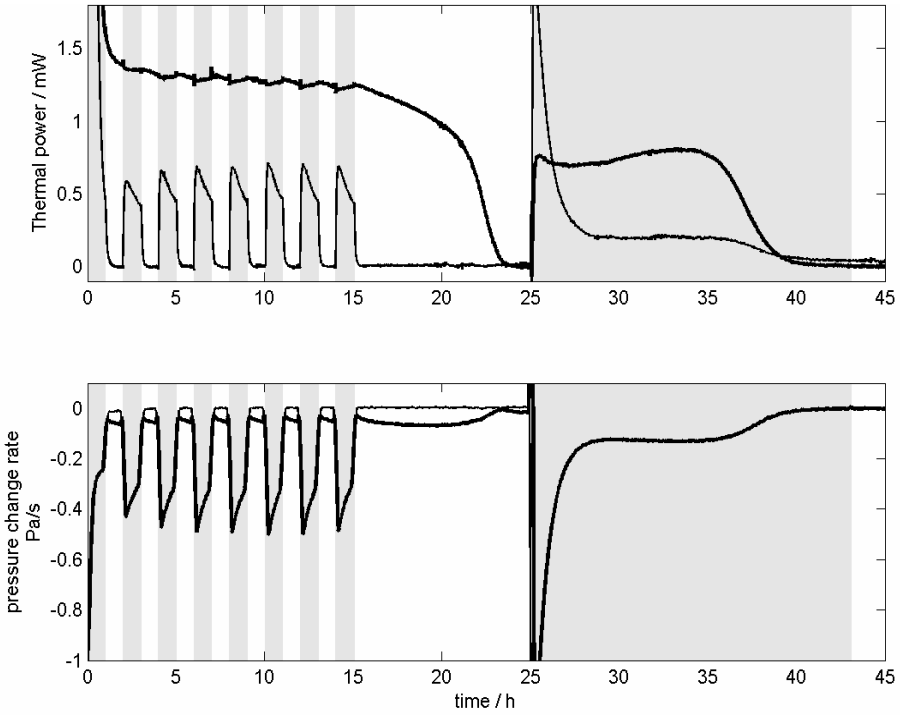

Figure 6.4 Results of measurement on *S. lacrymans* growing on 2% MEA. Top: thermal power from sample (thick line) and absorbent (thin line); Bottom: Pressure change rate from in sample vial (thick line) and absorbent vial (thin line). Gray area is when valve was open; white area is when valve was closed.

A simulation program was made and used to investigate the gas diffusion in the calorespirometric device. This was simulated by dividing the gas phase of the instrument into four parts and calculating the diffusion (and resulting convection) of N_2, O_2 and CO_2, with an explicit difference method (it was assumed that there were no water vapour gradients in the system). As the model was rather rough the behaviour of the model was improved by adjusting the conductances to give a good fit to the result of a simple experiment without any sample, in which the valve was opened when there were CO_2 on the sample side, but no CO_2 on the absorbent side.

In one end of the model the sample consumed O_2 and produced CO_2 according to a model of the biological sample, and at the other end CO_2 was consumed by the absorbent. During a simulation the connection between the two central parts of the model switched from no transport when the valve was closed, to normal diffusion when the valve was open. The simulation program was written in MATLAB 6 (The Mathworks, Natick MA, USA).

One rationale for developing the simulation model was to investigate the diffusion of the gases in the instrument and the dynamic changes of gas concentrations at the sample. Depending on the geometry of the instrument, the CO_2 production rate of the sample and the valve cycle times, the CO_2 concentration at the sample can change from low to high values, or remain essentially constant. During the measurement of *S. lacrymans* sample the simulation showed that the CO_2 did not drop to zero during the open cycles. This was because the diffusion flow path was relatively long in the present instrument. However, it was difficult to make it much shorter as it connects two calorimeters that must be separated to decrease cross-talk.

The models of the biological systems used in the simulation program contain functions for P_s (Thermal power of sample, W), ϱ (metabolic gas exchange ratio, mol(O_2)/mol(CO_2)) and $\Delta_s H$ (enthalpy change, J/mol(CO_2)) (i.e., the rates of O_2 consumption, CO_2 production and heat production) as functions of CO_2 and O_2 pressures. As an example, P_s could be modelled as decreasing when the CO_2 pressure increases and when the O_2 pressure decreases. For the test measurement on a sample of the dry rot fungus *S. lacrymans* satisfactory results were achieved with the following model (Fig. 6.6 and Fig. 6.7):

$$P_s = f_C(p_{CO2}) \cdot f_O(p_{O2}) \cdot P_0$$
$$\varrho = 0.8$$
$$\Delta_s H = 467 \text{ kJ/mol}(CO_2),$$

Here, P_0 is the thermal power of the sample at atmospheric conditions, i.e. at the start of the measurement. It should be noted that P_0, includes the effect of biomass, nutritional status, etc. In the present experiment we are studying relative changes when environmental parameters change. The factors f_C and f_O model how the sample reacts to CO_2 and O_2 pressures. In the present model the effects of CO_2 and O_2 are separated. Fig. 6.5 shows how f_C and f_O depended on CO_2 and O_2 pressures, respectively. The organism was modelled as being able to do partial anaerobic metabolism (ϱ=0.8) as this gave the best fit to the experimental results.

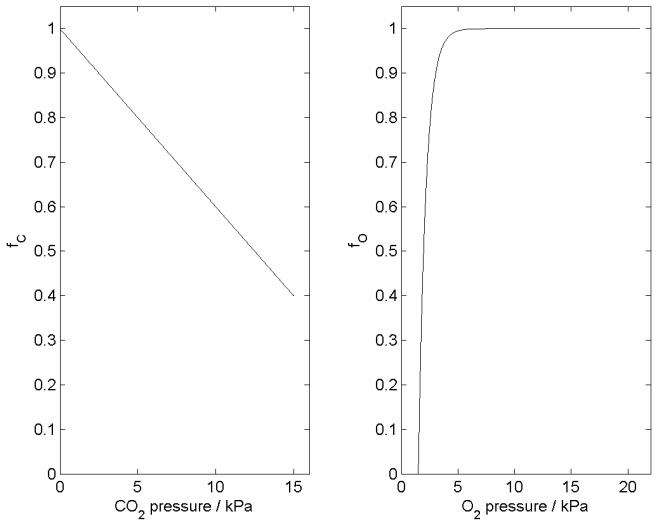

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Figure 6.5 Factors f_C and f_O used for simulation of *Serpula lacrymans* behaviour. The factors f_C and f_O takes into account the influence of CO_2 and O_2 pressure on the activity of a sample.

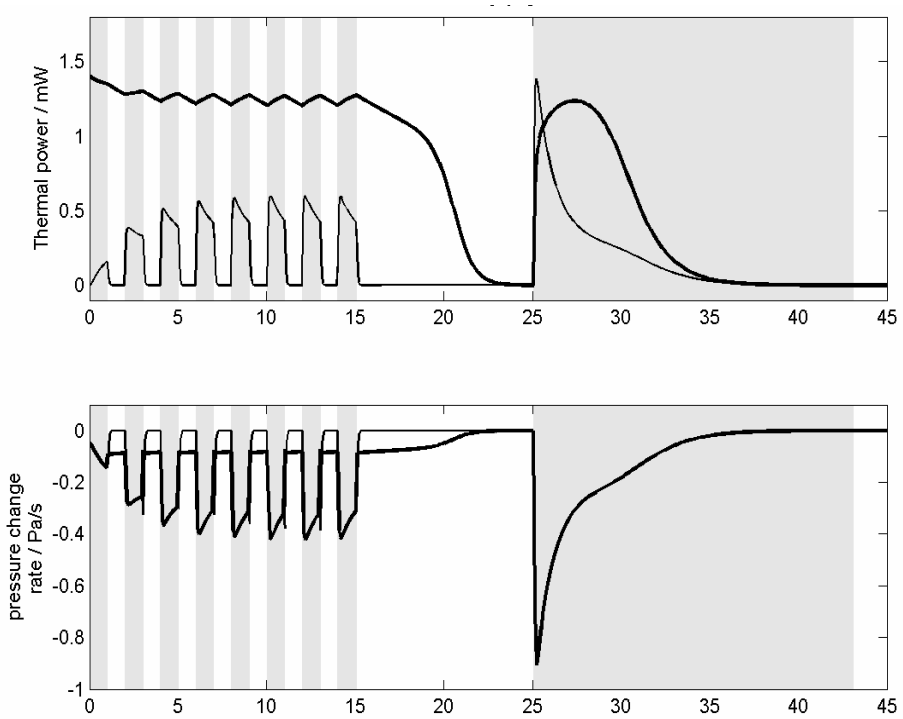

Figure 6.6 Simulated result of *S. lacrymans* growing on malt extract agar. Top: thermal power from sample (thick line) and absorbent (thin line); Bottom: Pressure change rate from in sample vial (thick line) and absorbent vial (thin line). Gray area is when valve was open; white area is when valve was closed.

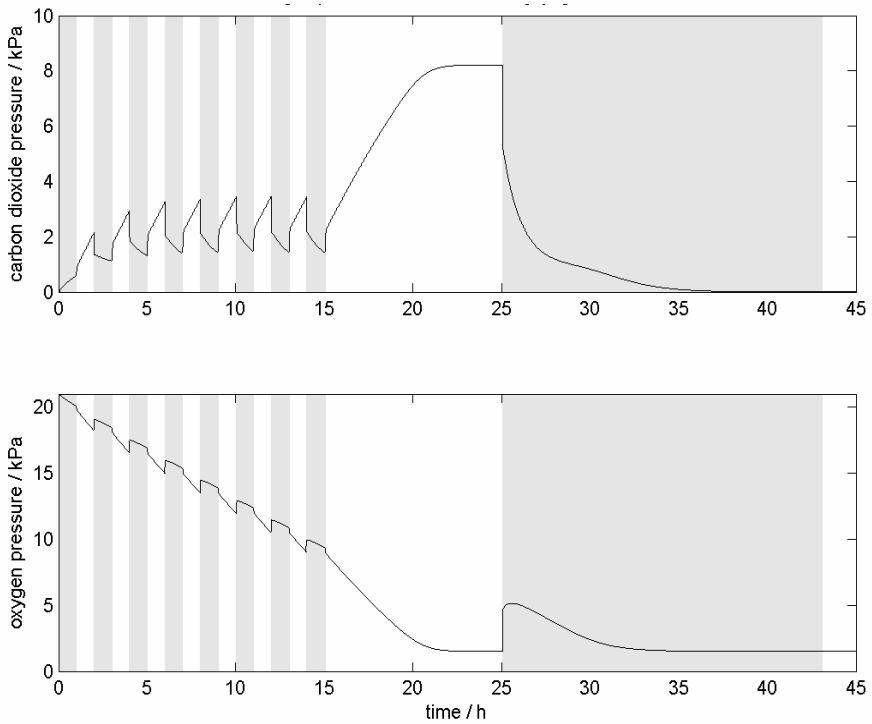

Figure 6.7 Simulated CO_2 (top) and O_2 (bottom) pressures for *S. lacrymans* growing on malt extract agar.

The comparison of the two mould fungi and one rot fungus are shown in Fig. 6.8. It shows that when oxygen pressure were above their critical level respectively their metabolic

enthalpies were kept relatively constant: about at 470 kJ/mol(CO_2). Their tolerances to low oxygen level were slightly different. *P. roqueforti* can tolerate lower oxygen pressure than *P. camemberti* which can tolerate better than *S. lacrymans*. This result is slightly surprising as rot fungi that grow inside wood are generally thought to be relatively tolerant to low oxygen and high carbon dioxide concentrations. However, *P. roqueforti* is specifically selected to grow inside cheese instead of growing on the surface only, which indicates it is not too dependent on high oxygen pressure as the ones growing on the surface only, e.g. *P. camemberti*.

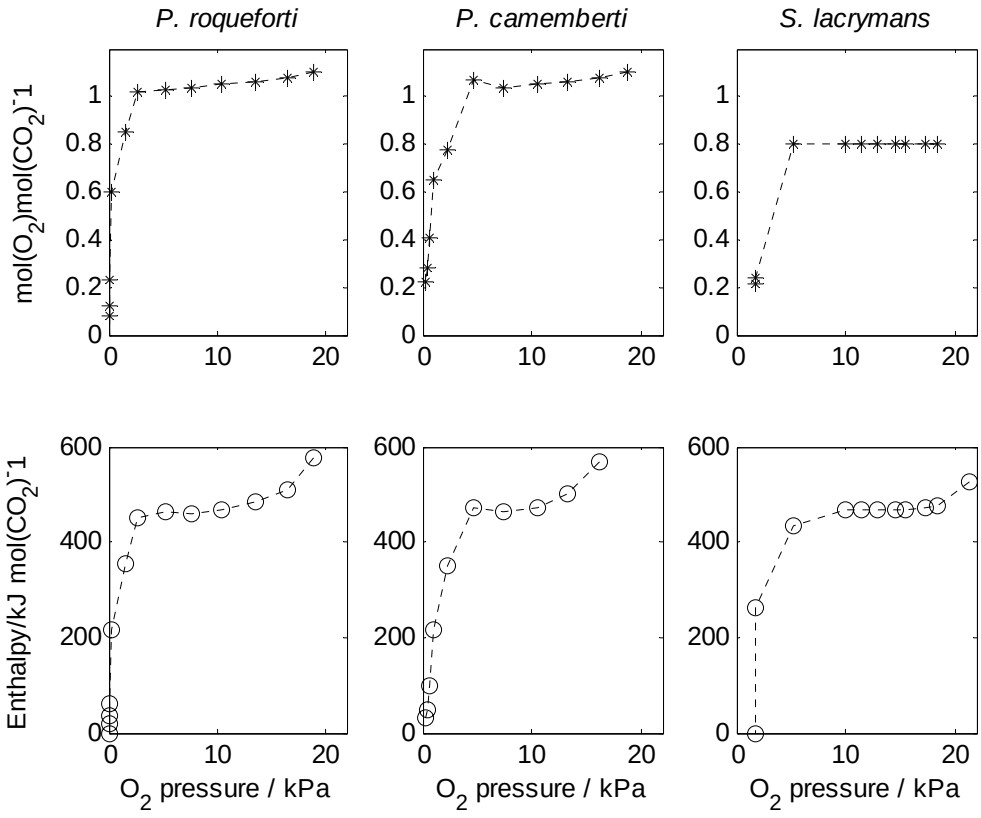

Figure 6.8 The gas exchange ratios (top) and enthalpy changes (bottom) as a function of oxygen pressure of mould fungi *P. roqueforti* (left) and *P. camemberti* (middle) and rot fungus *S. lacrymans* (right) growing on 2% MEA.

6.1.4 Conclusion

Results from both the mould fungi and the rot fungus showed that the activities of these fungi are typical of aerobic organisms, but not very sensitive to decreased oxygen pressure when it is above their threshold level (about 1-3 kPa). The enthalpy of the respiration was about 470 kJ/mol (CO_2), which fits Thornton's law. The gas exchange ratio (respiratory quotient) was about 1.0 for both of the mould fungi, which is not unexpected as the nutrients in the malt extract medium are mainly carbohydrates. The lower gas exchange ratio for *S. lacrymans* indicates that this rot fungus is not fully aerobic, but as this respiratory quotient was found from computer simulation it is uncertain and more measurements needs to be done.

6.2 Calorimetric measurements of fungal growth on agar

In any biological system, growth can be defined as the increase in cells numbers or the increase of all chemical components (EncyclopædiaBritannica 2007).

The growth of microorganisms such as bacteria and yeast are typically described in terms of biomass and as being in three phases: a lag phase, an exponential phase and a stationary phase (Fig. 6.9). Studies are then usually made by measuring the biomass of the microorganism culture grown in a shaken liquid medium (Carlile et al. 2001). The lag phase is the time it takes for the newly introduced organism to adapt to the new environment and start growing. The exponential phase refers to the period during which the biomass increases in an exponential fashion, for example the growth in a shaken liquid culture without any constraints for growth (e.g. nutrient, space, oxygen, etc.) except the organism's own ability to multiply. Then the organism divides at regular intervals and the growth becomes exponential (linear when drawn on a log-scale). The stationary phase refers to the period during which the growth of the organism slows down because of some constrains, e.g. depletion of nutrients. The biomass finally reaches a maximum level and stays constant (Carlile et al. 2001).

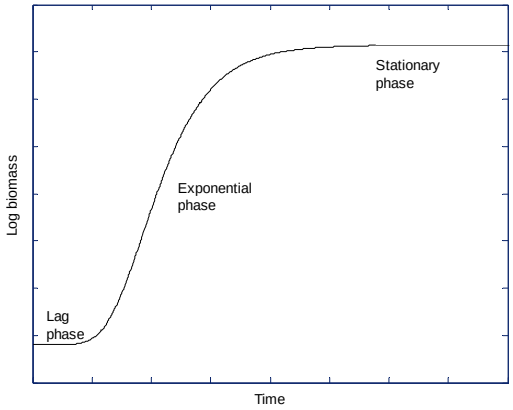

Figure 6.9 Growth phases of a batch culture.

Although the above "exponential model" of microbial growth is used in many microbiological studies, it is difficult to apply to the results of the studies of moulds growing on solid surfaces. This is mainly because the fungi do not multiply by division, but by hyphal extension in mainly two dimensions. There may also be diffusion limitations for the nutrients. For fungi growing on substrates like wood or a textile it is also difficult to measure the fungal biomass.

When growing on a surface with evenly-distributed nutrient, e.g. an agar surface, one has noted that the radius of a colony of mycelial fungi usually is proportional to time (not counting a lag phase and retarding/stationary phase) (Gonzalez et al. 1987; Valik et al. 1999), i.e. that the colony spreads outwards from the inoculum at a constant speed. If it is assumed that the biomass is proportional to the surface area of the colony such growth can be described by the following equation.

$$m(t) = at^2 \qquad (6.1)$$

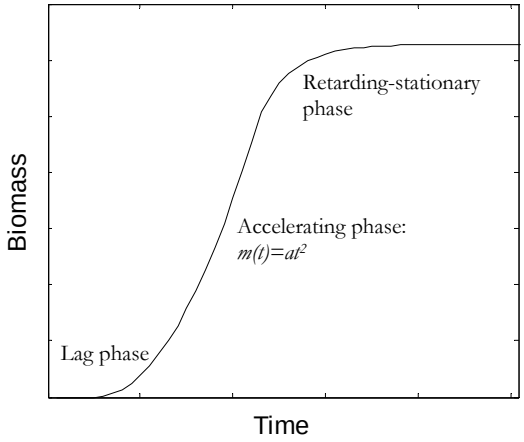

Figure 6.10 Simulation of biomass increase when growing on surface.

This fast growing phase is not exponential. The total growth from the inoculation till growth stops can be described as in Fig. 6.10.

One can look at fungal growth from different perspectives and describe it with different parameters. Biomass and colony diameter are two types of parameters commonly used. Fungal metabolism also changes during its growth: it increases with the increase of its active biomass and goes down when the fungus is dying. Therefore the thermal power, which reflects the level of fungal metabolism, can also reflect the fungal growth.

In paper II and IV the mould fungus *P. roqueforti* was inoculated with fresh spore suspension (a few thousand spores) in the middle of the substrate surface of 2 ml 2% MEA in 20 ml glass vials and then sealed with aluminium caps with Teflon-rubber septa. The vials were placed in a TAM Air (Thermometric AB, Järfälla, Sweden) immediately after inoculation and the measurement was started. During the measurement the vials were aerated 20 min (about 10-20 ml/min) every 12 hours with an aeration device providing sterile humidified air (about 100% RH) in order to avoid oxygen depletion or high concentration of carbon dioxide.

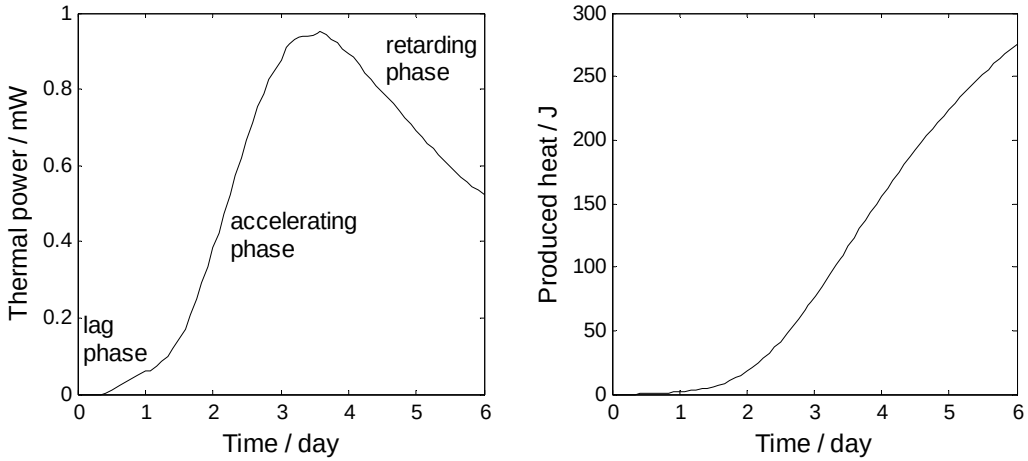

Figure 6.11 The three phases of fungal growth seen in calorimetric measurements of thermal power (left) and its corresponding heat produced. Measurement on *P. roqueforti* at 25˚C on 2% MEA.

Generally the thermal power and produced heat results from isothermal calorimetric measurements of surface cultures of mould growth look like in Fig. 6.11. Just as the growth of biomass or colony diameter can be divided into several phases, the calorimetric thermal power also clearly shows three phases: a lag phase, an accelerating phase and a retarding phase. The lag phase here corresponds to the lag phase described as in Fig. 6.10 and it is most probably the time it takes for the fungal spores to germinate and starts growing. After lag phase the thermal power increased rapidly (accelerating phase) although this is not an exponential growth since it is growing on a surface as discussed earlier in this chapter and in paper V. The accelerating thermal power can come from the accelerated biomass increase if it is assumed that the whole biomass is alive and active. Previous study also showed that there is a linear correlation between biomass and produced heat during this phase (Wadsö et al. 2004). After the accelerating phase, the increase of the thermal power decreases and finally the thermal power starts to go down. This period can be regarded as the retarding phase of the sample that is probably caused by nutrient depletion. At this phase the nutrient

in the agar may have been consumed and the fungi are using stored carbohydrates. The retarding phase of the thermal power indicates the fungal activity slows down while in measurements of biomass or colony diameter the retarding phase is an asymptote towards a constant value. Finally when all nutrient is consumed and the biomass is not alive anymore, the thermal power will become zero and the sample is no longer producing heat (not seen in Fig. 6.11).

The thermal power here is not a general index of biomass since not all the cells will have same metabolic level (biomass includes dead cells which have no metabolism and no heat production). Neither is the heat (integrated thermal power) a general index of biomass as maintenance heat can continue to be produced even if biomass is not increased. However, during the early accelerating phase when the whole biomass is alive the thermal power is an index of metabolism or activity of the sample. It is more complex than biomass measurements as the thermal power can (at least conceptually) be divided into maintenance and growth components (paper V). In this study, we use the measurement of thermal power as the index of fungal activity which is more connected to fungi's active growth.

6.3 Produced heat and ergosterol content of fungi

Calorimetry for measuring fungal activity is a relatively new method in fungal studies and it is quite different from the traditional methods, such as quantification of biomass, ATP, chitin or ergosterol. We found it important to correlate calorimetry with other techniques as a way to investigate the relation between calorimetry and other techniques and to get acceptance for this new method in fungal studies. Furthermore isothermal calorimetry can also provide additional information to the available methods. Therefore, the aim of papers II and IV was to compare and correlate three quite different methods to quantify fungal activity: heat production by isothermal calorimetry, biomass quantification and amount of ergosterol as determined by gas chromatography-tandem mass spectrometry (GC-MS/MS) (Li et al. 2007).

Ergosterol quantification is an established method in fungal research (Weete 1973; Weete and Laseter 1974; Grant and West 1986; Larsson and Larsson 2001). Ergosterol is a sterol that is found almost solely in the cell membranes of fungi (Seitz et al. 1977; Carlile et al. 2001; Newell 2001). It is thus well suited to be a unique chemical marker in fungal biomass estimation. The content of ergosterol in fungal mass is rather constant. A conversion factor of 5 mg ergosterol per g dry biomass is common in fungal research (Newell 2001).

In paper II three mould fungi and one rot fungus were investigated:

- *Penicillium roqueforti*
- *Cladosporium cladosporioides*
- *Neopetromyces muricatus*
- *Serpula lacrymans*

About 40 fungal samples of each strain were inoculated in 20 ml calorimetric glass vials with 2 ml 2% MEA and measured in a TAM Air (Thermometric AB, Järfälla, Sweden) at different stages of their growth. During the measurement, the samples were placed in the incubator which had the same temperature as during measurement, high humidity (>97%) and air exchanged through a filter. At each measurement stage, about three samples were removed

after calorimetric measurement, frozen and stored at –20°C for later ergosterol quantification. Ergosterol was quantified by gas chromatography-tandem mass spectrometry (GC-MS/MS) at the Section of Medical Microbiology, Lund University as by Sebastian and Larsson (2003). The results of the heat production, ergosterol quantification and the comparison between heat and ergosterol content are shown in Fig. 6.12.

The final ergosterol contents for every strain studied were similar (about 50 μg). This suggests that the total amount of ergosterol (or total amount of biomass) produced is related to the total amount of nutrients provided in the substrate. However, the heat production continued when the ergosterol level stayed constant. This heat was probably from maintenance metabolism only when the fungi used its own nutrients stored in the hyphae to keep alive or the fungi recycled its own ergosterol in its metabolism.

In those measurement results, we saw an initial approximate proportionality between heat and mass of ergosterol, which later shifted to a continued increase of heat while the mass of ergosterol stayed constant. From the initial rather linear slopes of the lines in Fig. 6.12 III the amount of heat produced per mass of ergosterol for different strains were calculated to be 0.8–5.4 J/μg (paper II).

The combination of two different methods: isothermal calorimetry in measuring produced heat and ergosterol quantification by GC-MS/MS gives interesting results for the understanding of fungal growth and metabolism. It was shown that the calorimetry can be combined or even correlated to the conventional mycological method.

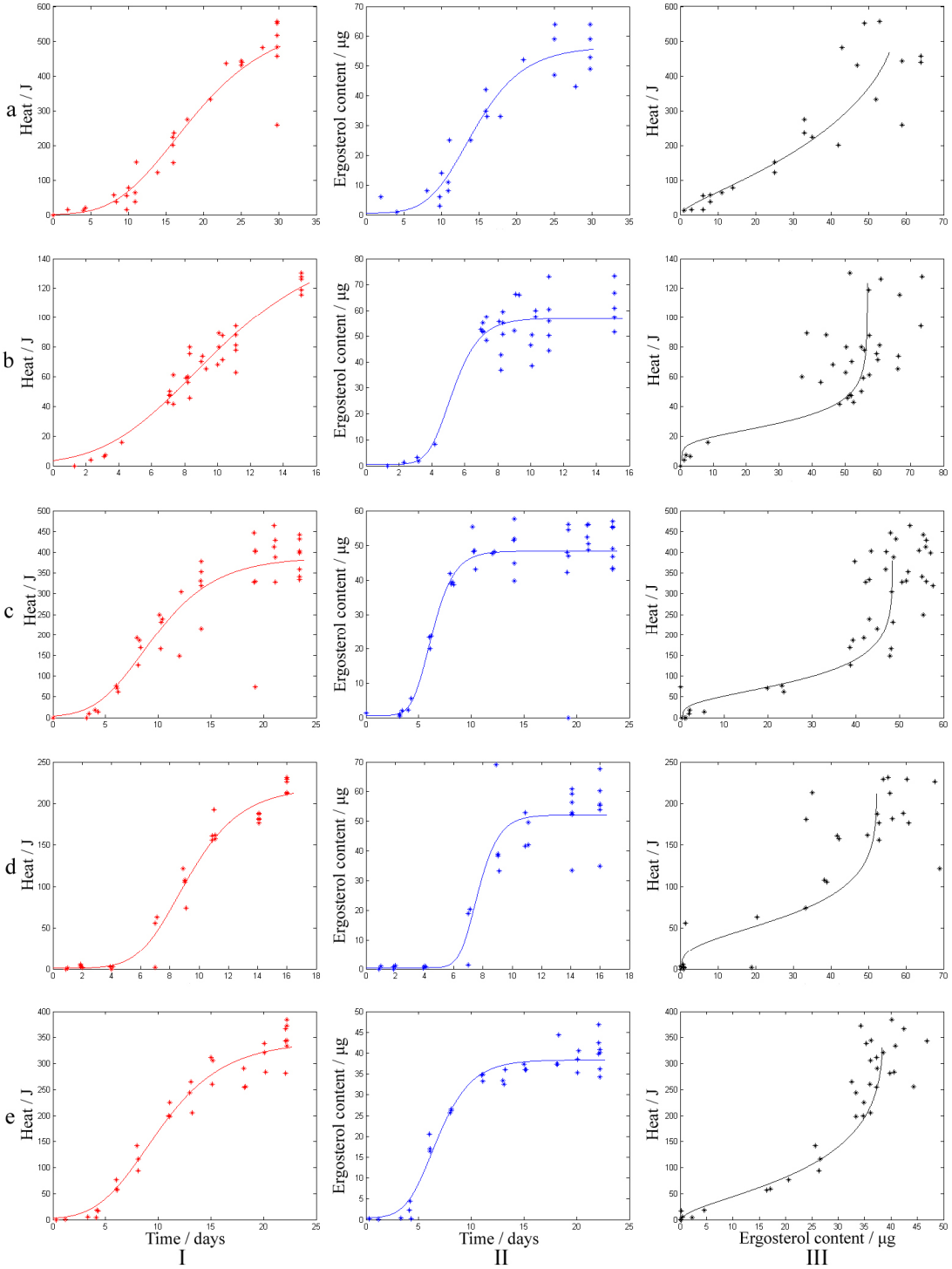

Figure 6.12 Results from paper II. I: total heat produced during fungal growth; II: ergosterol content of fungi during growth; III: total heat production vs. ergosterol content during fungal growth. a: *S. lacrymans*; b: *P. roqueforti* (20°C) c: *P. roqueforti* (15°C); d: *C. cladosporioides*; e: N. *muricatus*. All fitting curves are made using the Gompertz equation; the curve in III is the combination of the curves in I and II.

6.4 Impact of temperature on fungal metabolism

One of the most important factors for fungal growth is temperature. Since fungi cannot control their internal temperature, this is to a large extent influenced by the ambient temperature. Although fungi can grow in a relatively large temperature range (0-50°C) their growth rates are different at different temperatures. Normally, the temperature at which a mould has the fastest growth (rate of biomass increase) is accepted as the optimal temperature of that mould (Carlile et al. 2001). However, it is not well known whether this is also the temperature at which the fungi is growing most efficiently and under least stress.

Paper II (Li et al. 2007) showed that the heat production of fungi had linear correlation to its ergosterol content during the fast growing stage at constant temperature environment. It also indicated that even small temperature changes might influence the metabolic efficiency of fungi dramatically. In that study the mould *P. roqueforti* produced about four times higher amount of heat per unit of ergosterol produced at 15°C than at 20°C. An interpretation is that this mould fungus needs a higher amount of metabolism at the lower temperature to produce the same amount of biomass. The higher temperature 20°C is a more optimal temperature for this mould fungus since it was easier for it to produce biomass: it needed less 'work' for the same amount of production. As it is difficult to draw conclusions about how the temperature generally influences the fungal metabolism from the results of only two temperature levels, as in paper II, a study on mould metabolism in a wider range of temperatures was also made, with measurements of heat production, ergosterol content and biomass content both above and below the fungi's known optimum temperature (paper IV).

The mould fungus *P. roqueforti* was used for this study. Samples were inoculated with spore suspension in the middle of the agar surface in 20 ml glass vial which contains 2 ml 2% MEA. Each group is consists of 7 samples and one blank. There were in total five groups for the five different temperatures: 10, 15, 20, 25 and 30°C.

Samples were placed into a TAM Air immediately after inoculation and connected to an aeration system, which flushed humidified air through the vials for 20 min every 12 hours in order to prevent O_2 depletion or high concentrations of CO_2.

The calorimetric measurements were stopped during the fungi's accelerating stage which was determined from the thermal powers measured. The samples were then frozen at -20°C. Half of the samples were sent for ergosterol quantification with the same method as described in Chap. 6.3.1. The dry biomass of the rest of the samples were quantified with a sorption balance after freeze drying with a lyophilizer.

The rapid increase in heat production started much earlier for the samples at 20, 25 and 30°C than for the ones grown at 15°C and 10°C. The heat production increasing rate also varied at different temperature. It increased most rapidly at 25°C, followed by 20, 30, 15 and 10°C (Fig. 6.13). This suggests that the highest growth rate at 25°C for *P. roqueforti*.

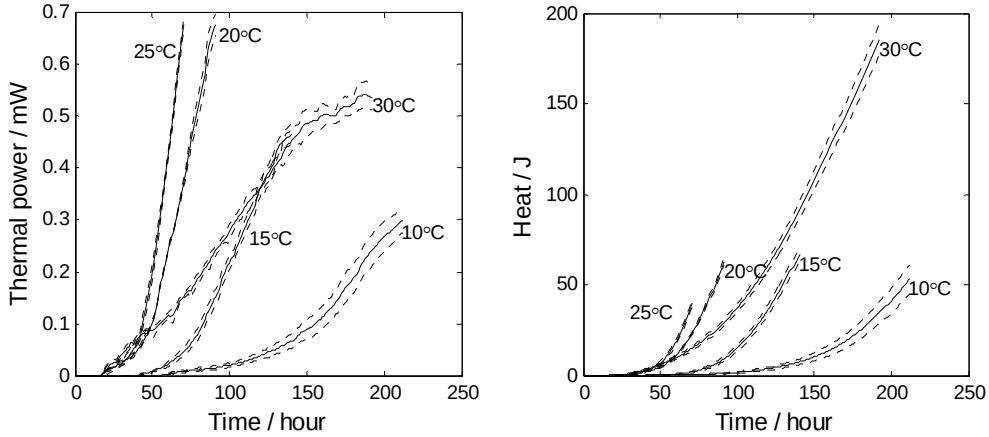

Figure 6.13 The thermal power (left) and produced heat (right) of *P. roqueforti* growing at different temperatures (mean value ± standard deviation).

The thermal powers of mould growth in these five different temperatures were modelled with the equations below (Paper V):

$$m(t) = at^2 \qquad (6.1)$$

$$P = \Delta h \cdot \frac{dm}{dt} \qquad (6.2)$$

Equation 6.1 indicates the biomass growth m (g) is proportional to the square of time t (as discussed in Chap. 6.2). Here a is a constant. The difference value of a indicates the growth rate. Equation 6.2 is based on that the thermal power P (W) measured is related to the rate dm/dt (g/s) of the biomass increase. Enthalpy change Δh (J/g) is included as proportionality constant. The simulated results of growth constant (a) and lag time at different temperatures are shown in Fig. 6.14. The lag time values also fit the time it took to start growth as seen in Fig. 6.13. The highest level of growth constant (a) indicates the highest growth rate at 25°C which agrees with the thermal power acceleration differences (paper V).

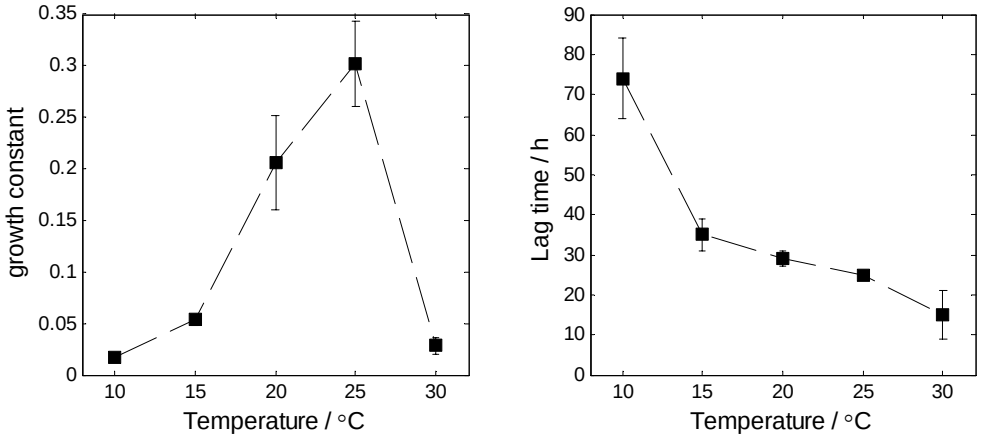

Figure 6.14 Simulation results of the mould growth constant (a) and the lag time of *P. roqueforti* growth at different temperatures.

The ratio between ergosterol mass and biomass was between 6 and 9 mg/g with highest mean ergosterol content at 20°C while it was lower when the temperatures were lower or higher than 20°C (Fig. 6.15a).

Previous studies suggested that environmental factors can influence the ergosterol content: it was lower in mycelia growing on low nutrient media and after moisture stress (West et al. 1987; Bjurman 1994), and it decreased in older cultures and after reduced aeration (Nout et al. 1987). Therefore the ergosterol content might be higher at conditions that are more suitable for the fungal growth (higher nutrient media, less moisture stress, better aeration, younger cultures, etc.) However the spread in the data was relatively high (biomass and ergosterol were measured on separate specimens from the same groups), therefore it is difficult to know if the trend given by the mean values is true. The lower range of this result is in good agreement with the generally accepted mean conversion factor from ergosterol to biomass, but all our results are within the range found in previous studies, see for example (Bjurman 1994).

The amount of total heat produced per unit of biomass or ergosterol content varied for the samples grown at different temperatures (Figs. 6.15b and 6.15c). It was lowest for the samples grown at 20°C. More heat was produced per unit of ergosterol content or biomass when the temperature was lower or higher than 20°C.

It has been convenient in this study to compare ratios, for example of heat and biomass. This is a way of not involving the rates of processes in the discussion. In the present measurements different amounts of heat were produced to make biomass at different temperatures. The highest amount of heat required to produce biomass (30°C) was three times higher than at 20°C. This rather large difference indicates that the formation of a unit of biomass needs more energy in the form of substrate at higher or lower temperature than at 20°C. The temperature at which the fungal metabolism is most efficient is thus around 20°C for *P. roqueforti* and not around 25°C where the highest (so called optimal) growth rates are found.

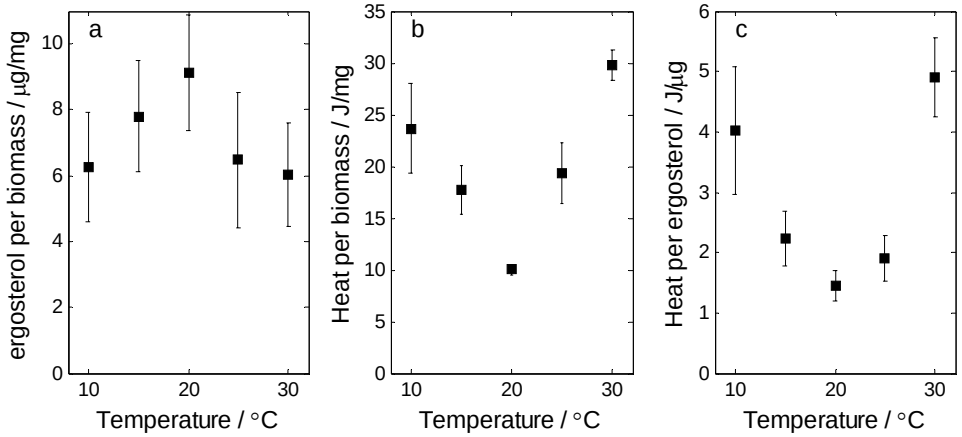

Figure 6.15 *P. roqueforti* at different temperatures: a. Ergosterol produced per unit biomass. b. Heat produced per unit of biomass. c. Heat produced per unit of ergosterol.

What is the optimum mould growth condition? Is it the level where the mould has most rapid growth or is it the level where the mould has the most efficient growth? These two

definitions may result in different conditions, as was shown in paper IV. However, one should not necessarily see a conflict here, since the mould growth itself is complicated and many factors are influencing the growth of fungi. The finding from this study may be useful for different purposes for further research, depending on if there is an unlimited supply of nutrients or not. For example, when it comes to buildings and indoor air quality, the level where the mould has most rapid growth might be the major concern since it is related to the fouling of building materials and the release of unwanted spores, MVOC, mycotoxins or mycelial fragments to the air. When it comes to the food industry, the most efficient growth may be more interesting since it concerns the conversion efficiency of production, e.g. fermentation.

6.5 Fungal activity as a function of moisture

The purpose of this study (paper VI) was to investigate the influence of moisture on the activity of mould growing on one of the most common building materials: wood. Wood can absorb substantial amounts of water vapour. At 100% relative humidity (water activity 1.0), the moisture content (mass water per mass dry material) of wood is about 30%.

In order to model the influence of the microclimate on the moisture state of mould fungi it is also of interest to know the sorption isotherm of the mould hyphae. As we measured sorption isotherms for wood for paper III we used the same method to also measure sorption isotherms for mould hyphae (not reported in the papers).

6.5.1 Sorption isotherm of fungal mycelia

The sorption isotherms of mycelia from one mould fungus *Penicillium roqueforti* and one rot fungus *Serpula lacrymans* were measured. About 4 mg of dry fungal mycelia were placed in a glass holder in DVS sorption device (DVS Advantage, Surface Measurement Systems Ltd. UK). The experiment started with the dry condition of the sample when the relative humidity was zero until the dry mass of the sample was determined. Then the relative humidity was increased stepwise from 0% till 95%. The mass of the sample were recorded for each level until it reached at relatively constant level. Afterwards the relative humidity decreased stepwise from 95% till 0%. The mass of the samples during desorption processes was also recorded (Fig. 6.16).

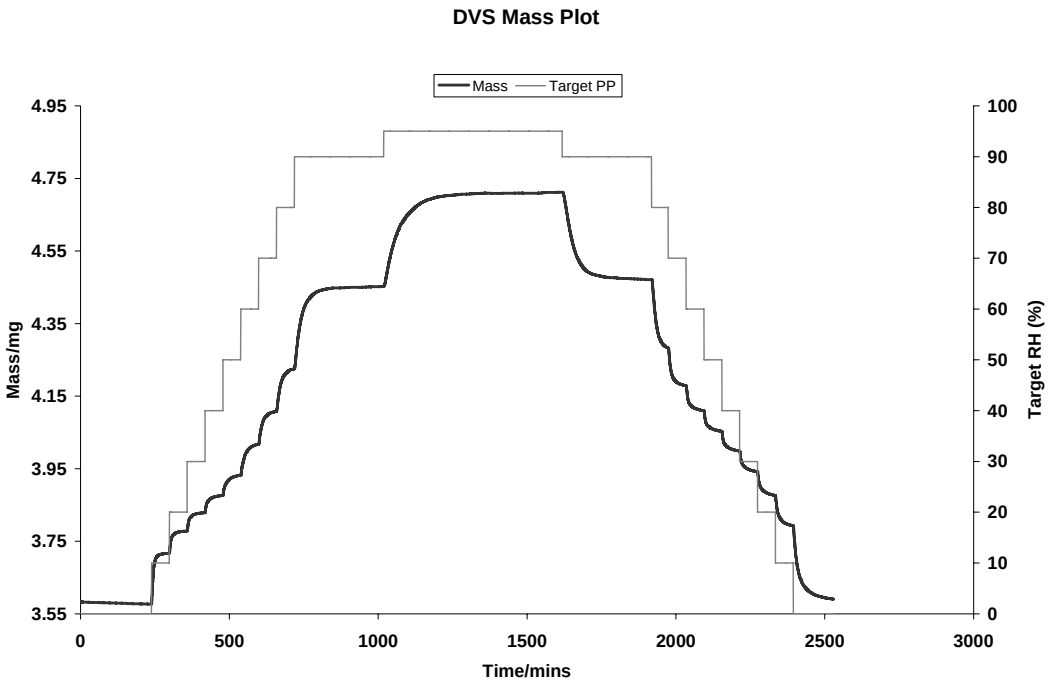

Figure 6.16 Relative humidity cycle for sorption measurements on fungal mycelia and the resulting sample mass. Target PP: target relative humidity.

The sorption curves of the mycelia of mould *P. roqueforti* (Fig. 6.17) and the rot fungus *S. lacrymans* (Fig. 6.18) showed similar trends in both absorption and desorption. They are also similar to, but slightly higher than the sorption isotherm of wood (Fig. 6.19). The moisture content of *S. lacrymans* was about 37% at 95% RH, which was higher than it of *P. roqueforti* which was about 32% at 95% RH. Note that these measurements were made on dead mycelia. It would be interesting to repeat the measurements on living mycelia.

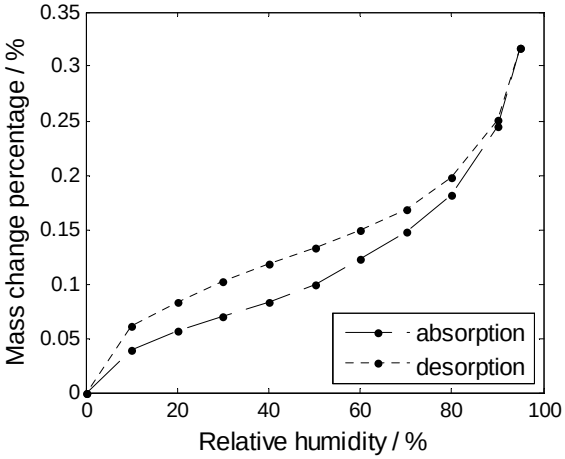

Figure 6.17 The sorption isotherm of mycelia of the mould fungus *P. roqueforti.*

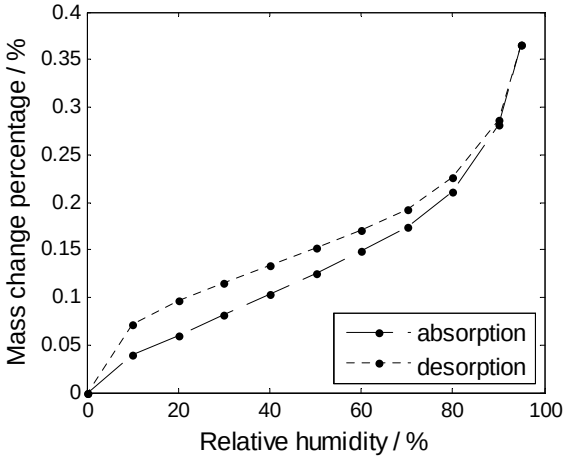

Figure 6.18 The sorption isotherm of mycelia of the rot fungus *S. lacrymans.*

The moisture content of fungi at a certain RH are slightly different depending on if that moisture state was reached by absorption or by desorption. This is interesting in relation to

what moisture parameter governs the activity of mould fungi. It is generally believed that water activity a_w or its equilibrium relative humidity (RH) is the most important moisture parameter for both biological and other processes, and this is certainly true if one compares different materials. However, looking at only one material or – as in this case – only one organism, it is possible that not only water activity, but also moisture content does influence reaction rates. At for example 70% RH there is a difference in moisture content of 2%-units between absorption and desorption (Fig. 6.17) and it is possible that this will influence the fungal metabolism even if the water activity is the same.

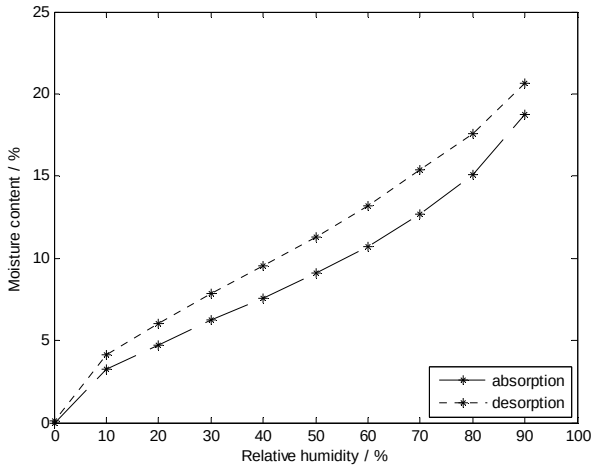

Figure 6.19 The sorption isotherm of wood (air dried spruce sapwood).

6.5.2 Mould fungus growing on wood

Previous studies have showed that mould growth on wood is only possible if its moisture content corresponds to a relative humidity (RH) higher than about 75%, depending on factors such as mould species, temperature, exposure time, etc. (Ayerst 1969; Grant et al. 1989; Wang 1994).

A preliminary study of how mould starts growing on wood by natural contamination in high humidity condition was made with calorimetry method.

Thin pine sapwood chips were exposed to room air and conditioned to water activity (a_w) of 0.95 and 0.90 respectively. They were placed in 4 ml glass vials (about 400 mg each) and sealed with aluminium cap with Teflon rubber septa. They were then placed in a TAM isothermal microcalorimeter for measurement. The samples were aerated with sterile humidified air with respective humidity level during the measurement. Their RH were measured and controlled during the measurement.

The thermal powers from the samples were zero at the beginning of the measurement (for about 2 days for the samples at 0.95 a_w and about 4 days for the samples at 0.90 a_w) before the thermal power accelerated rapidly (Fig. 6.20). It indicates that water damaged wood indoors or wood kept in high humidity will easily have mould growth. The higher a_w or water content will speed up the start of the growth.

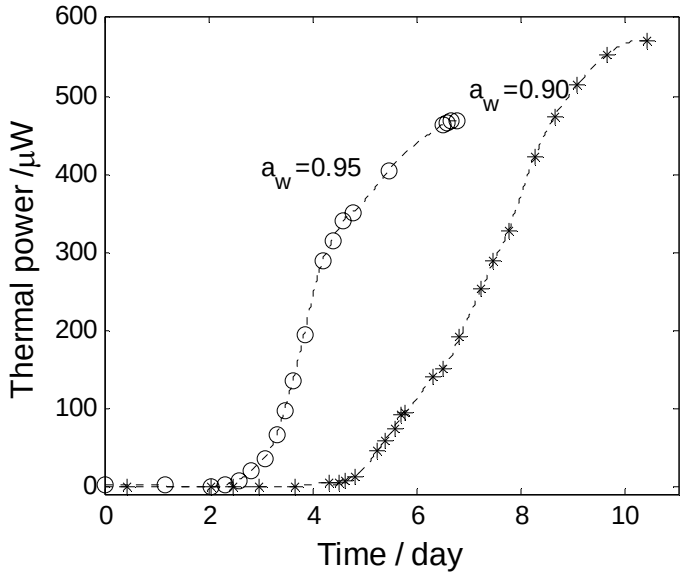

Figure 6.20 The start of mould growth on wood at high water activity levels.

Different relative humidity levels influences the mould growth (Ayerst 1969; Grant et al. 1989; Nielsen et al. 2004). How the RH influences the activity of mould growing on wood will be discussed in the next chapter.

6.5.3 Mould fungus growing on wood as a function of RH

The thermal activity of mould fungi growing on wood at different relative humidities will be discussed in this chapter (paper VI). Mould fungus used for this experiment was *Penicillium brevicompactum* (Strain nr CBS 119375). Wood used was pine (*P. sylvestris*) sapwood (paper VI).

Penicillium brevicompactum is a common indoor mould, which is also often found on wood materials (Bjurman 1994; Nielsen 2001). Spores of *P. brevicompactum* was collected from one week-old colonies growing on 2% malt exact agar (MEA) and dissolved in tween solution. The spore suspension was evenly spread on the surface of 2% MEA agar in 80 ml glass Petri dishes and then incubated at room temperature (about 20°C) in high humidity (>97% RH) for one week.

Wood samples were taken from a board with a yellowish surface, indicating that it was rich in nutrients (Theander et al. 1993). Thin wood samples (1 mm x 5 mm x 10 mm, mass about 50 mg) were cut so that one of their largest surfaces contained the drying surface. Before inoculation they were sterilized by γ-radiation (20 kGy) at the Radiation Research Department, Risø National Laboratory in Denmark. After sterilization, the pinewood had a moisture content of about 0.1 (water mass/dry wood mass). Sterile deionized water was added to specimen and the water content of the specimens was adjusted to be 0.3, which is equivalent to a water activity close to 1.0 for wood.

Two wood sticks were placed on the top of the mould growth surface in the Petri dishes with one week old *P. brevicompactum* growth. Test specimens were placed on top of the long wood sticks in order not to have direct contact with the mould surface or the agar (Fig. 6.21).

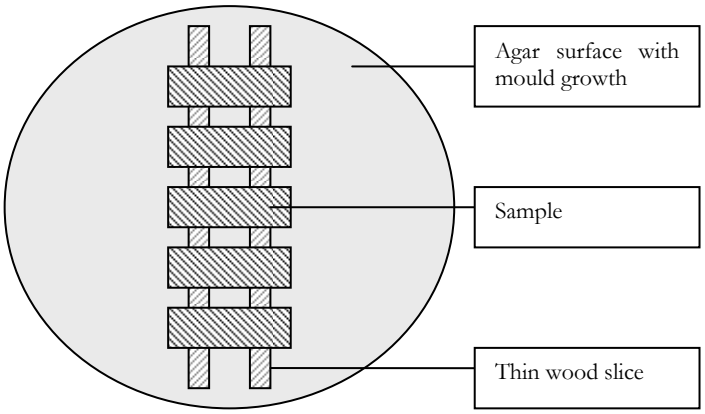

Figure 6.21 Schematic picture of the incubation of test specimens.

The samples were then collected two weeks after incubation and placed in 4 ml glass vials and sealed with aluminium caps with Teflon rubber septa. Each vial contained about four pieces of wood samples. The thermal powers from the vials were measured with an isothermal microcalorimeter and the moisture content of the samples was modified with an external humidity generator. The relative humidity and the mass of the samples were measured every time after the calorimetric measurements.

The water content of the specimens was measured by recording the mass of the samples after each measurement and comparing to its dry mass (105°C for 12 hours) measured at the end of the experiment.

In these measurements the wood samples were exposed to step-wise changing moisture conditions. At each step, both relative humidity and moisture content were quantified, and it is thus possible to construct a sorption isotherm for the wood materials. This is shown in Fig. 6.22 for one of the samples.

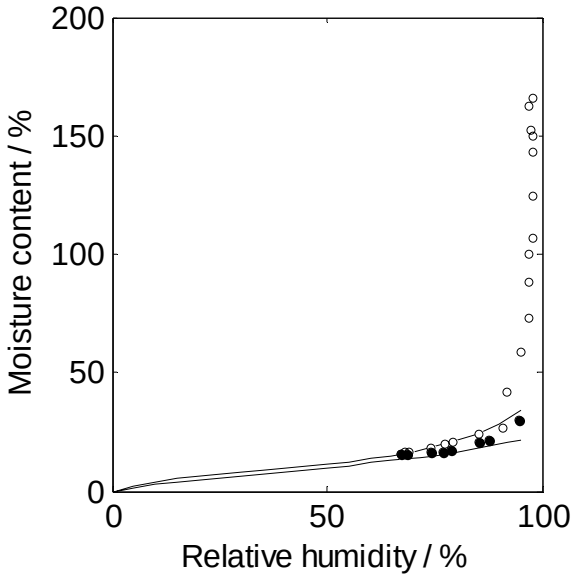

Figure 6.22 Sorption isotherms calculated from the present measurements (circles, white are for desorption, black are for absorption). The solid line shows results from measurements on spruce wood.

It is seen that the results follow a typical wood sorption isotherm, but that the moisture content was far up in the super-hygroscopic region when the measurement started. The results in Fig. 6.22 show that the method used to change the water state of the samples worked well. The measurements should, however, preferably be started with less excess of free water as it took a long time to remove this water.

All thermal power measurements made showed qualitatively similar results. First high thermal powers at high moisture levels; a decreased thermal power as the moisture content was decreased; a significant transient increase at about 30% moisture content; low levels at low water activities; and finally an increase in thermal power at the end of the measurements when the water activity again was increased. The highest (at least transient) fungal thermal activity was at a water activity level close to 0.92 while the fungal activity was lower at higher water activity (0.95).This agrees with Grant and co-workers' finding that *P. brevicompactum* has highest growth rate close to 0.88 but lower at higher levels when growing on malt extract agar (1989). The thermal power measurement was plotted together with relative humidity and moisture content in Figure 6.23.

The thermal power as a function of RH during desorption and absorption for one of the samples is plotted in Fig. 6.24 (not included in paper VI). The thermal power was taken 90 min after the sample was inserted into the calorimeter. The lines are subjectively drawn trend lines. The differences in thermal power between desorption and absorption are clear.

According to the nature of hydrophilic materials, the water content is higher during desorption than during absorption (at the same water activity). This might explain, at least partly, why the mould activities are lower during the absorption process, and that biological activity is not only a function of water activity, but also of moisture content. A similar conclusion was reached by Labuza and co-workers for lipid oxidation in food-stuffs (1972). This is contrary to the present concept that water activity is the proper water quantity to use when investigating the relation between water state and reaction/growth rate (Scott 1957). It is probable that when looking at different samples (with different sorption properties) the water activity is the best parameter to use, but when looking at only one material the moisture content (hysteresis limb) is also of importance.

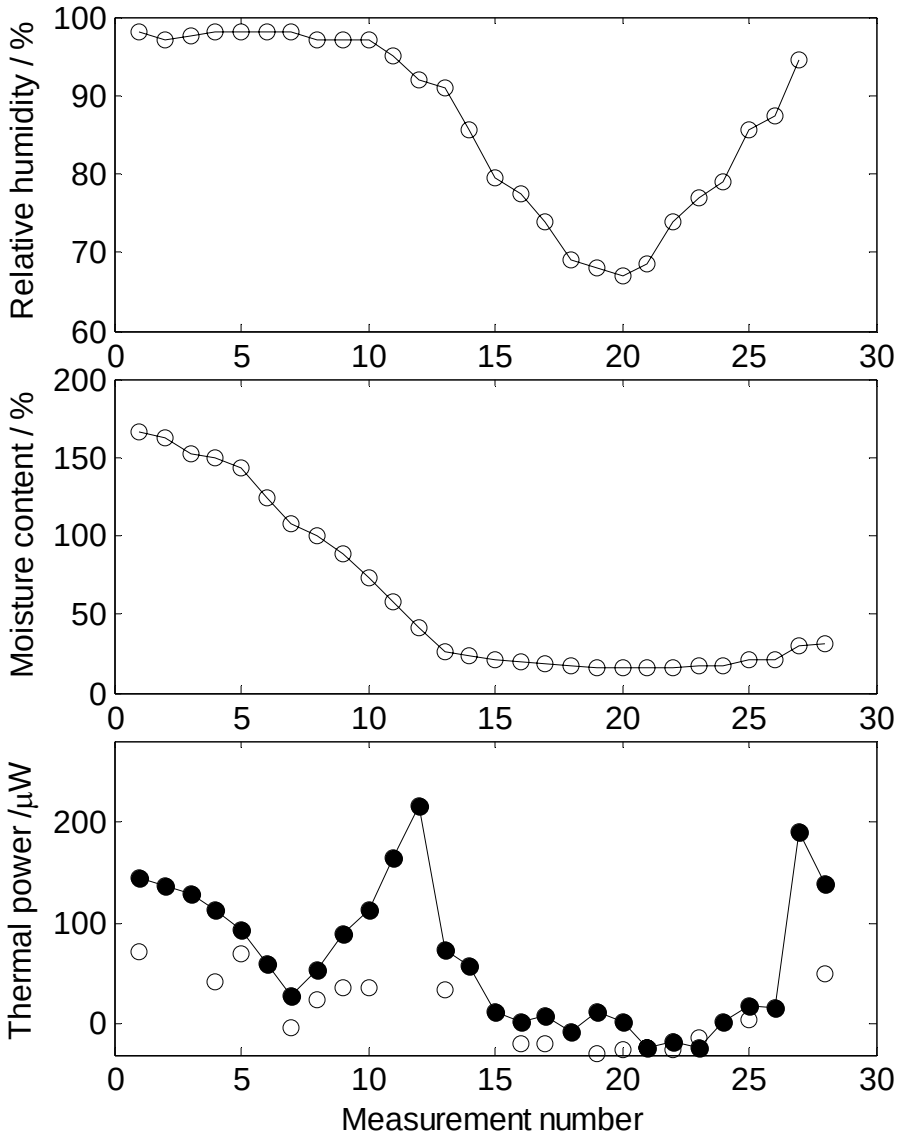

Figure 6.23 Overview of the results for one sample of the mould *P. brevicompactum* growing on wood during a desorption-absorption cycle. Each point corresponds to one humidity level. In the bottom figure the black markers are mean values between 100 and 150 min after a sample was charged into the calorimeter. The white markers are final values for the humidity levels where the measurement continued for such a long time that it was possible to evaluate a final constant thermal power level.

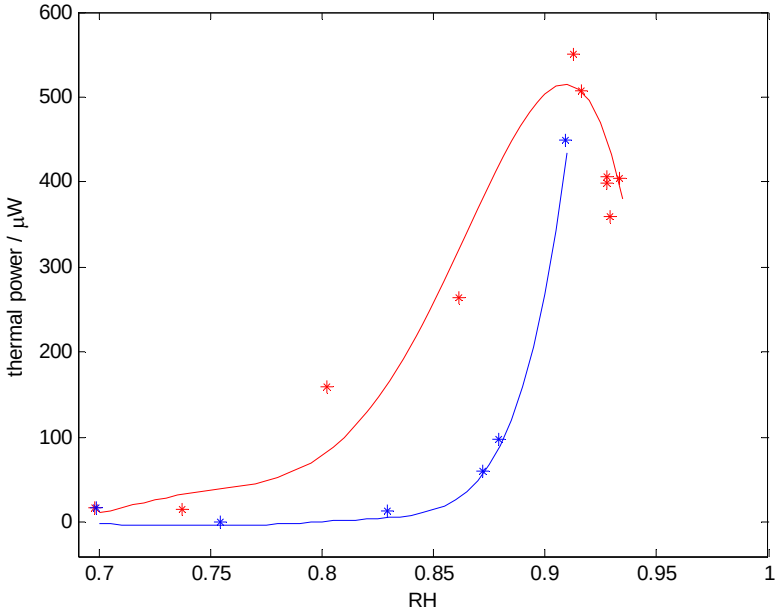

Figure 6.24 Thermal power of *P. brevicompactum* on pine wood under different relative humidity. (red: during desorption, blue: during absorption)

The results of the measurements showed that isothermal calorimetry has potential for measuring mould fungal activity as a function of RH, water content (and then also of temperature). However, the results of these measurements (paper VI) were not trivial to interpret as the calorimetric signal seldom reached constant values, and in some cases negative values were measured. It is probable that there are moisture sorption related heat productions superimposed on the biological heat production. This should be further investigated.

If these measurement problems can be solved, the advantage with calorimetry over most other used techniques is obvious: it is a non-destructive method that can easily be applied to measure fungal activity of mould fungi on real building materials. In order to achieve a more complete modelling of mould behaviour on building materials, future research can be done with more calorimetric measurements on:

- More indoor mould species (pure and mixture)
- Different temperature/relative humidity levels
- Different materials (both organic and inorganic)
- Dynamic changes of temperature and relative humidity conditions (basing on the dynamic building environment conditions)

Chapter 7

Mould growing on heat treated wood

Wood is one of the most common building materials used in most climates. Mould easily grows on wood when it is moisture or water damaged. For example moulds appeared about one week after water damaged wood was used in a house (Fig. 7.1). Wood can be infected when in the forest or during storage. When conditions are favourable, mould can grow in different parts of the building where it is applied: roof, attic, walls, furniture, floor and etc.

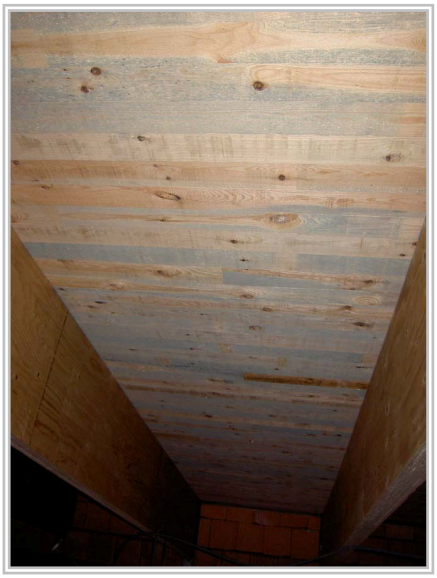

Figure 7.1 Mould growth on water damaged wood in a building in Skåne, Sweden

The most common wood species used in the Nordic construction industry is Norway spruce. However, the use of larch is increasing in exposed constructions such as panelling and outdoor decking as this wood is believed to be more durable because of its high heartwood content.

Normally wood has to be dried before use in order to increase the strength and reduce its water content for easier transportation and better preservation. There are many different drying methods; the most common one is kiln drying where water is removed by air convection. Wood can be dried at different temperatures depending on species, final application, dryer facility and time available. Normally it is believed that drying reduces the water content and thereby reduces the risk for mould growth.

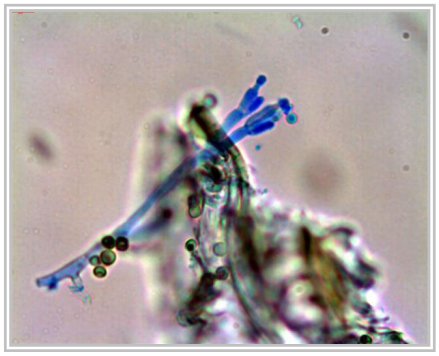

Figure 7.2 Microscopy picture of two moulds on wood samples from a damaged building (Fig. 7.1). The blue hypha are *Penicillium* and the green-brown spores are *Cladosporium*.

However, the risk for mould growth still exists or even will be increased if dried wood is exposed to high relative humidity. One possible reason is that sugar and other nutrients are transported to the surface during the

drying process. Several studies have found that wood dried with fast schedules had higher risk for mould growth than slowly dried wood (Terziev et al. 1993; Theander et al. 1993; Viitanen 2001). The risk for mould growth is also related to the drying temperature. One study showed that high-temperature-dried wood had less mould growth (Sehlstedt-Persson 1995). However, only two temperature levels (115°C and 65°C) were compared in that study.

In this study (paper III), the risk for mould growth on spruce and larch dried at different temperature levels was investigated.

7.1 Mould susceptibility of heat treated wood

7.1.1 Material and method

The wood used in this investigation consisted of Norway spruce (*Picea abies*), Hybrid larch (*Larix x eurolepis*), European larch (*Larix decidua*) and Siberian larch (*Larix sibirica*). Sticks with dimensions 20 x 40 x 300 mm^3 were sawn from both heartwood and sapwood of frozen logs and dried to target moisture content of 6% in a climate chamber at 80, 120 or 170°C. Spruce dried at 20°C was air-dried in a climate room at 20°C and 30% RH until constant weight. After drying, the specimens were stored in a climate controlled room at 20°C and 60% RH. The heat-treated material was sawn into 20 x 40 x 300 mm^3 sticks and conditioned at 20°C and 60% RH. Together with the Thermowood® (obtained from Stora Enso's heat-treatment plant in Kotka, Finland) there are in total 6 different temperature treatment levels investigated in this study: 20, 80, 120, 170, 190 and 210°C.

When conditioned, specimens with approximate dimensions 40 x 100 x 5 mm^3 were sawn from the original drying surface of the sticks. Each specimen thus contains one original drying (or heat treatment) surface and one re-sawn surface located 5 mm below the original drying surface. This was done because it has been reported that nutrients are transported to the surface during drying so that mould growth is heavier on such surfaces.

Besides the natural exposure of the wood samples during storage, two additional mould species were used: *Cladosporium cladosporioides* and *Penicillium commune*, both of which were isolated from a piece of wood taken from a house in Skåne, the southernmost part of Sweden (Fig. 7.1). Mould samples were grown on 2% malt extract agar (MEA) before inoculation.

In order not to alter the wood properties, the samples were not sterilized. One week before inoculation, all specimens were placed in plastic boxes at 20°C with nearly 100% RH to increase moisture content in order to facilitate spore germination (Fig. 7.3). The sample was separated into two groups:

- Natural contamination group. The specimens were exposed to indoor air before the investigation; therefore they have "natural" contamination with mould spores from the indoor air.
- Natural and controlled contamination group. A mixed spore suspension of *Cladosporium cladosporioides* and *Penicillium commune* (about $1x10^4$ spores/ml) was sprayed evenly on both sides of the specimens in addition to the natural exposure

during storage. About 0.4 ml of spore suspension, i.e., about 4000 spores were sprayed on each side of the specimens in this group.

Between four and seven specimens per species-temperature-combination were assigned to each of the groups. All specimens were placed directly on top of a plastic net in standing position in plastic boxes with water underneath, which provides a relative humidity close to 100% during the experiment and incubated for 20 weeks.

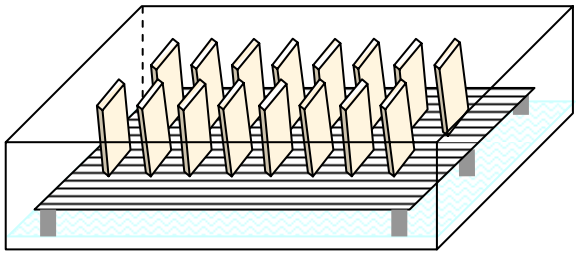

Figure 7.3 Incubation of wood samples in moisture environment.

7.1.2 Image analysis

Each sample was photographed on both sides (original drying surface and re-sawn surface) once a week during the experiment with a digital camera (Nikon D70s). After one week at 100% RH, the specimens were photographed for the first time and then inoculated. Therefore, the first week only natural contamination is recorded. At the end of the experiment, all samples were inspected with light microscopy and photographed with 400x magnification.

The mould growth during the 20 weeks of incubation was quantified by image analysis with the following steps:

1. The jpg-images were transformed into grey scale by taking the mean of the red-green-blue colour levels (Fig. 7.4).
2. As the aim was to quantify the darkening of the specimens caused by dark mould growth the grey scale was inverted so that 0 was white and 255 black.
3. The grey levels of three reference areas outside the specimens (lines on the photographic table) were quantified. This was necessary as the light intensity in the room was not constant during the 20 weeks of the experiment.
4. The grey level of each specimen was measured as the mean intensity of a rectangular box covering about 80% of each specimen (so as not to introduce any edge effects).
5. The darkening *d* (0: white; 1: black) of a specimen at week *i* was calculated as:

$$d = \frac{D_s(i) - D_r(i) - (D_s(1) - D_r(1))}{256} \qquad (7.1)$$

Here, D is the grey level of sample (s) and reference area (r) on the white to black 0-255 scale. There are two corrections made to the grey level value $D_s(i)$. First the grey level of the reference areas $D_r(i)$ is subtracted to account for that the lighting in the room changed during the experiment period. Secondly, the grey level of the sample before any mould growth occurred (at week one) was subtracted to correct for the original colour of the wood sample. The image analysis was made with a program written in MATLAB.

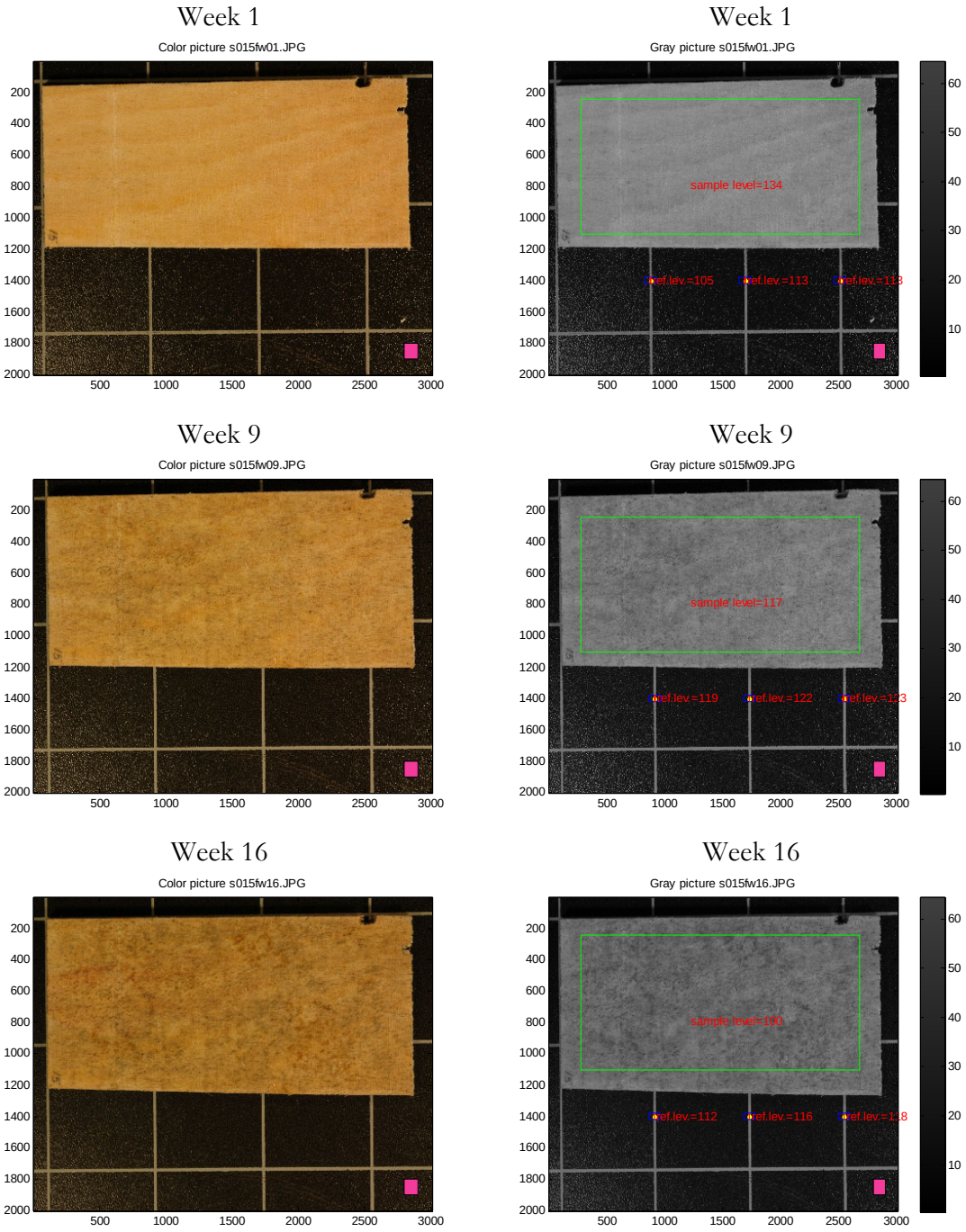

Figure 7.4 Example of image analysis of one of the specimens (spruce sapwood, dried at 80°C, original drying surface) at week 1, 9 and 16. To the left are colour photographs and to the right are the corresponding gray picture with the reference level areas automatically found by the image analysis software.

7.1.3 Sorption isotherm

Sorption isotherms were measured on thin shavings of early wood of spruce. The samples had dry masses in the range 3.1-5.5 mg. The measurements were made in a DVS Advantage sorption balance (Surface Measurement System Ltd., UK).

7.1.4 Results

The sorption isotherms were about the same for wood (spruce) dried at temperatures up to 170°C. The heat-treated samples (190°C and 210°C) had substantially lower sorption isotherms (Fig. 7.5).

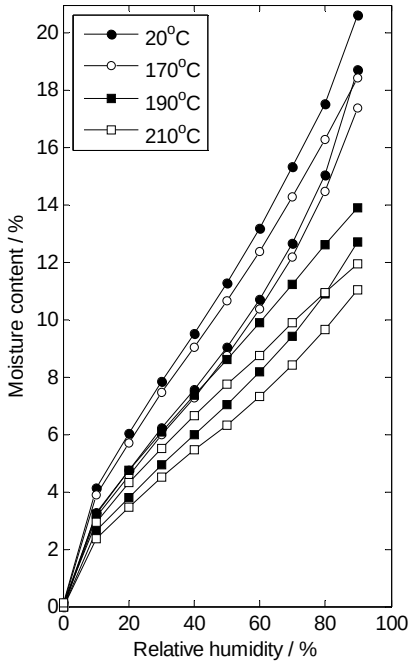

Figure 7.5 Results from sorption isotherm measurements on spruce early wood treated at different temperatures. For each temperature the lower line is for absorption and the upper line is for desorption. The results at 95% RH are not shown as they did not attain equilibrium.

Mould growth started at week two on most of the specimens that were not heat-treated. After week 13 the rate of increase of the mould coverage slowed down (Figures 7.4, 7.5 and 7.7). This agrees qualitatively with Viitanen and Ritschkoff's (1991).

The mould growth on most specimens dried up to 170°C with natural + controlled contaminations was higher than the growth levels on specimens with natural contamination only, cf. Fig. 7.6., This difference between natural and natural + controlled contamination was not significant On heat-treated wood.

Mould growth on the original drying surface was in most cases higher than the growth on the re-sawn surface (Fig 7.6 and 7.7). The reason for these differences is most probably the relocation and accumulation of nutrients at the surface during the drying process as suggested by previous studies (Terziev et al. 1993; Theander et al. 1993; Viitanen 2001). During the drying process, soluble nutrients are transported with the water towards the drying surface where water is evaporated. Non-volatile nutrients are then left on the surface or close to it. Differences in mould growth on original and re-sawn surfaces were observed

both for natural contamination and for natural + controlled contamination. However, the mould growth on both sides of the heat-treated specimens (190 and 210°C) was low and the differences were not significant.

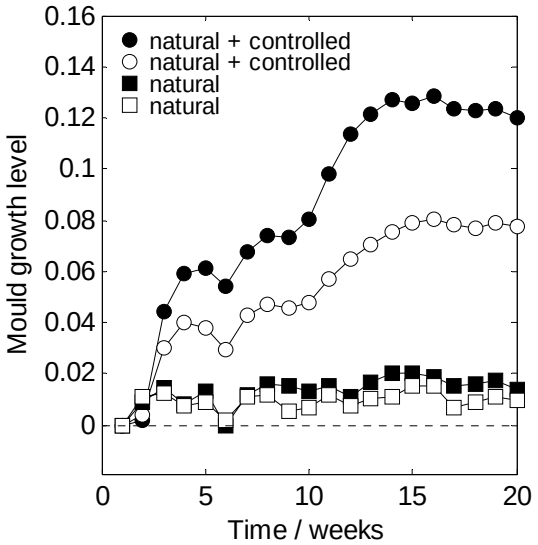

Figure 7.6 Example of mould growth levels on air dried spruce specimen from image analysis. ●,■: Original surface; ○, □: re-sawn surface.

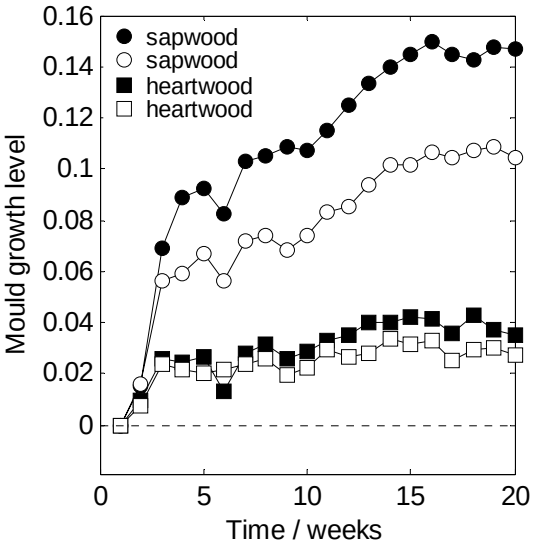

Figure 7.7 Example of mould growth levels from image analysis on heartwood and sapwood of spruce dried at 80°C (natural + controlled contamination) ●,■: Original surface; ○, □: re-sawn surface.

The mould growth level on sapwood was in general higher than it was on heartwood, as is shown in Fig. 7.8. The different levels of mould growth on sapwood and heartwood with different temperature treatments for spruce are shown in Fig. 7.8. One reason for the differences between sapwood and heartwood might be that the nutrient level in sapwood is higher than in heartwood as found by other studies (e.g. Viitanen 2001). Furthermore, heartwood extractives might be inhibiting the fungal growth (Philip et al. 1995). For heat-treated samples (190°C and 210°C), no significant difference in mould growth was found between heartwood and sapwood.

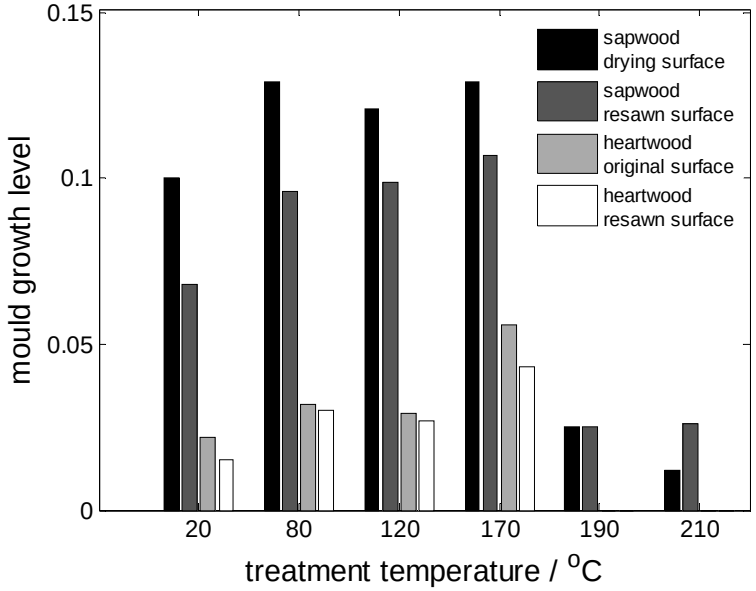

Figure 7.8 Average mould growth levels on spruce from image analysis after 20 weeks.

Mould growth was most rapid for specimens dried at 80°C, cf. Fig. 7.9. After about 9 weeks, 80°C-specimens had highest mould growth, followed by samples dried at 170°C, 120°C and 20°C. However, after 20 weeks, all kiln-dried specimens reached a similarly high mould growth level which was higher than for the air-dried sample (20°C), cf. Figs. 7.8 and 7.9. The heat-treated samples had very low mould growth during the 20 weeks of the measurement. They were difficult to inspect visually and due to the dark colour of heat-treated wood the image analysis also had difficulties in assessing the mould growth on these specimens. However, microscopy after the measurement revealed that there was only very sparse mould growth on all of the heat-treated specimens.

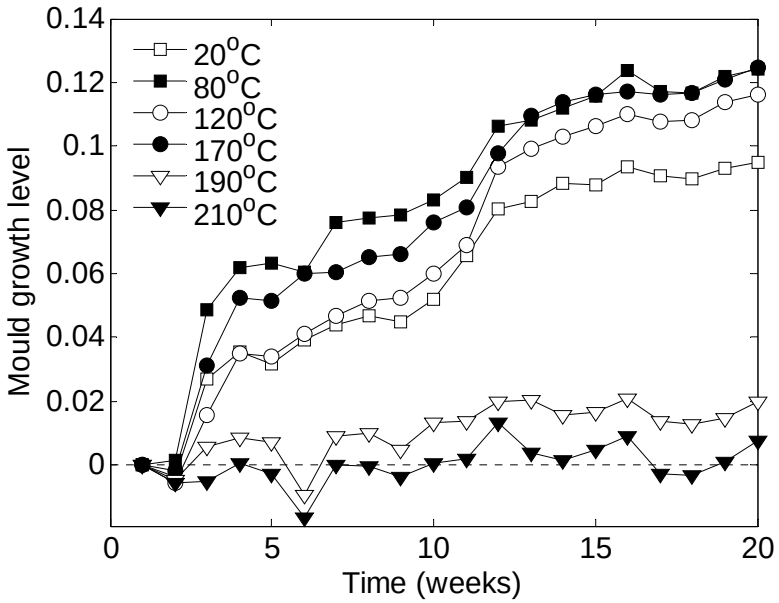

Figure 7.9 Average mould growth levels on spruce sapwood from image analysis. Specimens had natural + controlled contamination and the results shown are for the original drying surfaces.

The reason for the higher mould growth levels in kiln-dried samples is probably the transport of nutrients from the core to the surface during the drying process. Air-drying at 20°C was very slow and probably less nutrient transport occurred. Low mould growth levels in the heat-treated samples are probably caused by altered properties of wood, such as the degradation of nutrients or the formation of toxic compounds.

Note that the relatively lower mould growth level in the air-dried wood does not imply that air-drying is the preferred drying method since it is time consuming and thus expensive. In the present experiments air-drying was done in a climate room at low relative humidity (30%) and low temperature (20°C), and with a probably very low exposure to mould spores. If air-drying is made outdoors the climate will not be as dry as in our study and there will in most cases be a heavy contamination with mould spores.

Heat-treated wood has high resistance to mould growth. However, as the heat-treatment also makes the wood more brittle, it is not recommended to use heat-treated wood for load-bearing purposes. However, for such uses as unpainted outdoor panelling it may be an interesting alternative to kiln-dried wood.

In the present study, image analysis was used to evaluate the mould growth level instead of only visual inspection as is usual. This gave quantitative results that qualitatively agreed with our visual observations. Image analysis is less subjective than visual inspection and has the advantage of evaluating the density of mould growth which is difficult to do by traditional visual inspection. However, the method may need to be optimized so that the influence of specimen colour and texture does not influence the results. In the present study the image analysis did not work satisfactorily for Russian Siberian larch which was dark and had a distinctly waxy surface. It may also be a problem that the wood progressively gets darker when it absorbs water vapour as it was doing in the present study.

Further studies on other wood species might help to understand the impact of drying method on mould growth. Further work should also aim at finding optimum methods, as e.g. optimum temperature levels or different drying methods as e.g. contact drying. Also wood modification leading to decreased availability of water and nutrients to the mould or water storage, leading to leakage of nutrients could result in decreased risk for mould growth.

Chapter 8

Conclusion

Although calorimetry is a relatively new method in fungal studies, it has been shown to give unique and useful information on fungal growth. Calorimetry has several advantages compared to traditionally used techniques to study fungal growth and activity: it is non-destructive, continuous and not limited by sample/substrate properties and shape. In some respects it is related to other used techniques; for example is the heat produced proportional to the biomass during the accelerating phase of growth on an agar. In other respects does calorimetry give new information; for example the thermal power during the retarding phase indicates the maintenance metabolism based on the use of stored nutrients.

The most important findings in this project were as follows:

1. An improved calorespirometric method was developed. This was shown to be able to simultaneously measure thermal power, oxygen depletion and carbon dioxide production. The fungi investigated were shown to have different lower limits for oxygen pressure.

2. The relation between ergosterol content and produced heat was investigated for several fungi. It was seen that they were proportional during the accelerating phase, while at the retarding/stationary phase the heat production continued while ergosterol levels remained constant. Thus calorimetric measurements on samples that have depleted their external nutrient supply can quantify the maintenance metabolism when the fungi uses internal stored nutrients.

3. The relations between heat and the two commonly used fungal growth indices, ergosterol content and biomass, were determined at five temperatures for *P. roqueforti*. It was found that the ratios heat/ergosterol and heat/biomass were temperature dependent.

4. From values of heat/biomass metabolic efficiencies can be calculated. Such calculations were made and showed that the growth efficiency of *P. roqueforti* was higher at 20°C, than at 15 and 25°C.

5. It was shown that the most efficient growth temperature does not equal to the temperature of highest growth rate. For *P. roqueforti* these were 20 and 25°C, respectively (measurements were made in 5 degree intervals).

6. The calorimetric measurements at different temperatures were fitted to several theoretical growth equations with time lags. It was found that even if the calorimetric results are nearly continuous and thus contain more information than point measurements, it was difficult to find a single equation that had the best fit as all equations could fit the results reasonably well. Different time lags were obtained with different methods. Examples of the equations with more fitting parameters but less unique solutions were also given.

7. A calorimetric study of how the moisture state influences mould growth was also made. This indicated that mould growth was higher at around 92% RH, than at higher or lower humidity levels. It also indicated that mould growth shows a hysteresis similar to sorption hysteresis, i.e. it produces higher thermal powers at a certain water activity when in desorption than in absorption. However, these results should be seen as preliminary as the measurements were difficult to interpret, probably because thermal power from water sorption/redistribution was superimposed on the biological thermal power.

8. A study of mould development of wood dried/treated at different temperatures was also made. The image analysis technique to quantify mould growth developed worked well when there was a clear colour difference between mould and wood.

Generally the two quantitative methods used in this work – calorimetry and image analysis – were satisfying. It was shown that calorimetry is not an independent method but correlated to other methods of studying fungal growth. However, calorimetry is also unique in providing information on fungal metabolism. The perspectives can also be much broadened when calorimetric method and other biological methods are combined.

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