"BIODÄM"
Examinations of the behavior of classical and ecological insulation materials concerning microbial contamination at different climatic conditions - assessment of the influence on indoors-hygiene and the efficiency of energy-saving measures

Aim

Within the framework of the project "Biodäm"[1] selected classical and ecological insulation materials were tested for their liability against infestation with moulds. Furthermore it was assayed, to what extent the insulation characteristics of the tested materials would be changed by such microbial infestation.
Therefore the insulation materials were incubated in a climatic chamber, with changing temperatures and rising air humidity. The microbial infestation on the material surfaces and pore voids was analyzed microscopically and microbiologically.
Thereby it was decisive to gather as well the presence of microorganisms as their actual metabolic activity on the insulation material by using suitable procedures.
After microbial infestation the changes in building physical parameters (thermal conductivity and water vapour permeability) on the contaminated insulation materials were analyzed.
Following these results an assessment should be given concerning the possible occurrence of hygienic pollution.

Execution

Following insulation materials were examined:

mineralic	mineral wool expanded perlite expanded clay foamed glas
synthetic	polystyrene expanded (EPS) polystyrene extruded (XPS) polyurethane foam (PU)
ecological	cellulose fiber wood fiber cork flax fiber

The insulation materials were stored under controlled climatic conditions at standard climate (20 °C, 65 % relative humidity) before the tests.

Following moulds were used:

Aspergillus fumigatus
Aspergillus niger
Aspergillus terreus
Aspergillus versicolor
Aureobasidium pullulans
Chaetomium globosum
Epicoccum nigrum
Penicillium chrysogenum
Stachybotrys chartarum
Trichoderma viride

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A spore-solution was made from these fungi (number of spores in solution 10^6/ml). With this spore-solution the samples were contaminated.

For taxonomic examinations a slice of agar was put onto the sample´s surface. This agar slice was incubated in a petri-dish afterwards and the growing moulds were characterized taxonomically.

Microbiological Examinations

The incubation was done in a climatic chamber. Every three parallels of each insulation material were incubated with a not inoculated blank. The incubation temperatures were 16 °C, 20 °C, 22 °C and 24 °C, respectively. The air humidity was 40 % rel. hum., weekly increased by steps of 5 % to final 90 % rel. hum.

fig. 1: climatic chamber with sample arrays for the tests at different temperatures and air-humidities.

As a rule daily macroscopic or microscopic examinations were done. The metabolic activity was measured weekly by determination of ATP-content of a defined surface area. For the definition of biodegradation the formation of carbon dioxid was measured via gaschromatography for selected insulation materials. A test for inhibitors (agar-inhibition-test and test following the guide-lines of DIN IEC 68) was performed to detect possible biocidic impregnation of the insulation materials.

Building-physical examinations

For the determination of thermal conductivity plate-formed square sample-bodies with an edge-length of 500 and 200 mm, respectively, were used. The material was dried before testing at 70 °C until reaching constance of weight. After the measurement of thermal conductivity at dry and wet condition, the samples were sprayed with a spore-solution. An incubation at room-temperature and rel.hum. of 90 % for 12 weeks followed. Afterwards the thermal conductivity was measured again.

The measurements of water vapour permeability were done following the guidelines of DIN EN ISO 12572 (2001). After the first measurement, the samples were sprayed with the spore-solution and incubated for 12 weeks in the climatic chamber at 20 °C and 90 % rel. hum. After this time water vapour permeability was measured again.

Results

The **ecological insulation materials** tested (esp. wood fibre and cork) were extremely suspectible to infestation with moulds, already at moderate temperatures (20 °C) and

increased air humidity (75 %). The high **hydrophobicity** of wood fibre and cork was not influenced by fungal growth. The values of **thermal conductivity** for flax and wood fibre were negatively changed by ongrowing fungi. The values of **water vapour permeability** for flax and wood fibre were, even in the state of fungal contamination, within the framework of values citated in literature; therefore it can be said, that these changes of values do not mean a loss of insulation properties. The water vapour permeability however was obviously not changed by infestation with fungi.
In contrast to the other tested ecological insulation materials **cellulose** showed to be to a large extent resistent against microbial contamination. An inhibiting effect was proven in an agar-inhibition-test, which was confirmed by the inhibition-test following the guidelines of DIN IEC 68 without extra nutrients. This lead to the conclusion that biozides must have been added to the insulation material to inhibit the growth of moulds (probably borates).

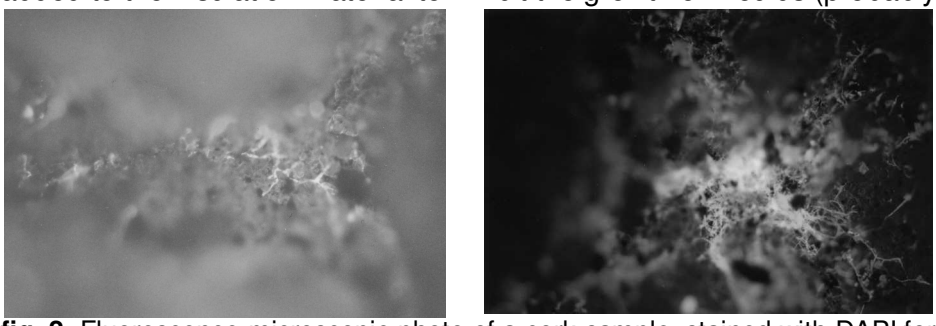

fig. 2: Fluorescence-microscopic photo of a cork-sample, stained with DAPI for illustration of microbial contamination (left, after incubation at 22 °C, magnification 100 times) and with Acridinorange (right, after incubation at 24 °C, magnification 100 times).

For the **mineralic insulation** materials a higher temperature (22 °C) and a longer residence time under conditions of the highest air humidity (90 %) was necessary to promote growth of moulds on the materials. Of these materials only mineral wool and expanded clay were suspectible to infestation with moulds. The **hydrophobicity** of mineral wool was, as for the ecological insulation materials cork and wood fibre, very high. The contamination with moulds did not change the insulation characteristics.

The **synthetic insulation materials** (EPS, XPS) were at temperatures up to 24 °C to a large extent inert against infestation with moulds. At higher temperatures (24 °C) growth of moulds on these insulation materials occured. Astonishingly this occurred already at low air humidity (45 %). It can be definetly excluded, that the growth of moulds was caused only by this low air humidity. It is more propable, that the fluid, which was applied to the insulation material's surface by spore-solution, faciliates the growth of fungi. The high **hydrophobicity** of synthetic insulation materials when not infested decreased with increasing incubation temperature and therefore increasing contamination with moulds. Therefore it can be said that hydrophobicity is considerably influenced by growth of fungi. The **water vapour permeability** of XPS increased when contaminated with moulds, which will in practise leed to a slighter diffusion of humidity. Nevertheless the values do range within the limits of literature-values.

Polyurethane foam (PU-coated with cellulose) was a special case: Although it is a synthetic insulation material, it is on the surface coated with a cellulose-containing layer, which was very attractive to microorganisms. It was, as the ecological insulation materials, already infested at 20 °C. At 24 °C it was, as the other synthetic insulation materials EPS and XPS, already grown with moulds at very low air humidity (45 %). As for these insulation materials the hydrophobicity of PU decreased with increasing temperature, if fungi grew on. Water vapour permeability was strongly increased in the course of fungal growth. It was probably caused by a destruction of the cellulose-coating on the PU, whereas this one becomes more permeable. The heavy infestation with fungi in contrast to the slight infestation on the ecological insulation material cellulose may be caused by a not exisisting biozidic coating.

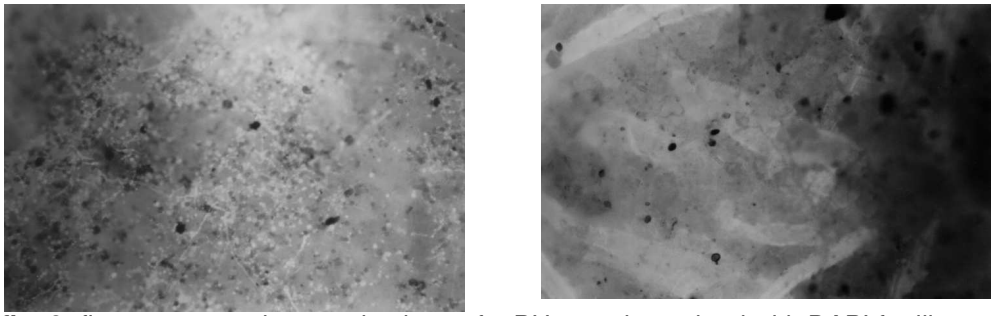

fig. 3: fluorescence-microscopic photo of a PU-sample, stained with DAPI for illustration of microbial contamination (left, after incubation at 22 °C, magnification 32times) and with Acridinorange (right, after incubation at 24 °C, magnification 32times).

Tab. 2: Overview of values for thermal conductivity, water vapour permeability and macroscopic visible contamination with moulds on the insulation materials

insulation material	**thermal conductivity • * [W/m·K]**		**water vapour permeability μ***		**contamination with moulds -intensity- (macroscopic)**
	before	**after contamination**	**before**	**after contamination**	
mineral wool	0,035	0,035	3	6	0
expanded perlite	0,040	0,040	19	14	0
expanded clay	0,050	0,050	5	14	0
foamed glas	0,040	0,040	10	12	0
EPS	0,035	0,035	24	30	+
XPS	0,035	0,035	56	155	+
PU (laminated with cellulose)	0,035	0,035	157	93	+
cellulose fibre	0,040	0,040	3	7	+
wood fibre	0,040	0,050	3	6	++
cork	0,045	0,045	13	39	++
flax fibre	0,050	0,055	6	6	+++

*values rounded

The colonisation of the synthetic insulation materials at 45 % rel. air humidity can in our opinion be put down to the fact that the spore-solution (fluid) remains on the materials surface for a relatively long time. These insulation materials are the only ones with a very plane and hydrophobic surface. Therefore the applied spore-solution can not seep into the material or dispense on it. So it could be possible, that the fungi, in spite of the very low air humidity, have enough humidity at disposal to start growing. As soon as this humidity is used up – which will probably occur rather rapidly at such low air humidity – growth ends. This can also be seen at the relatively small growth of fungi at low air humidity compared to higher air humidity. In any case, there is no question about the fact, that at such a low air humidity (as 45 %) a growth of fungi only caused by this air humidity is NOT possible. It can only occur, if at least for the start of growth a high enough humidity (higher than the air humidity) is given.

In generally it can be concluded, that temperatures higher than 20 °C and air humidity higher than 75 % favour the growth of moulds on ecological insulation materials, on some of the mineralic insulation materials and few of the synthetic insulation materials.

The taxonomic examinations showed a conspicuous occurrence of *Aspergillus fumigatus* on almost every sample at 22 °C. This was in contrast to the results at 24 °C. Several fungi were identified, which had not been part of the inoculum. Especially *Penicillium decumbens* and

Trichoderma viride seem to be obviously typical wood-populating fungi. At 22 °C it was especially *Stachybotrys atra* growing on wood fibre, besides this organism seemed to be a colonizer of sythetic insulation materials at 24 °C. Cork and wood fibre already seemed to be contaminated with fungal spores in delivery condition, which was not too astonishing, as the production of these insulation materials will not be carried out under sterile conditions. At 22°C wood fibre favoured the growth of the potentially pathogenic fungus *Stachybotrys atra*. At 24 °C wood fibre and PU favour the growth of the potentially pathogenic fungi *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus versicolor*.
Concerning the judgement of the taxonomic identification of fungi, it has to be considered that a distorted image of the infestation is given, as all fungi-material lying on the surface of the sample is taken off. So it is possible that fungi grow on agar, but need not necessarily grow on the sample. Nevertheless it is the easiest, fastest and most practicable method to analyze a contamination with fungi on a sample.

Tab. 1: Overview of isolated moulds on the insulation materials at 22 °C and 24 °C and classification into grade of endangering [Kähler 2000]

	expanded clay	**mineral wool**	**cork**	**wood fibre**	**PU**	**XPS**	**EPS**	**wood fibre-blind sample**	**risk group (corresponding BG Chemie 1997)**
inserted moulds									
Aspergillus niger	22			24	24				1
A. terreus			22	24					1
A. versicolor				24	22			24	2
A. fumigatus		22	22	22	22			22	2
Penicillium chrysogenum	22			24					n.s.
Trichoderma viride	22		22						n.s.
Chaetomium globosum		22					22		n.s.
Stachybotrys atra				22					n.s.
Epicoccum nigrum		22							n.s.
Aureobasidium pullulans									n.s.
other isolated moulds									
Aspergillus clavatus	22								n.s.
A. niveus		24		24					n.s.
A. paradoxus		24							n.s.
A. hollandicus							24		n.s.
Penicillium decumbens			22						n.s.
P. glabrum			22	24				22	n.s.
Cladosporium cladosporoides				24					n.s.
Eurotium amstelodami				24		24	24		n.s.
E. quadrilineata					24				n.s.

risk group 1: mould with which there was not yet reported an endangering for healthy persons (no or very low risk)
risk group 2: mould which can cause mycoses in healthy persons or persons with minor defections of immun-response. For medicamentation effective medicine exists.
n.s. (not specified): these moulds are not mentioned in the list
22/24: mould was isolated at this temperature

Assessment of results for practical use

A contamination with moulds may occur on almost every material, if the environmental conditions are adequate. From the ecological point of view they are decomposers, as they degrade ecological compounds and by this recycle nutrients. Thus mould-spores are found ubiquitarily in the environment [LGA-Bericht 2001, Mücke & Lemmen 1999]. Moulds live saprophytic and grow on decomposable material. Spore-germination, growth of mycelium, and spore-generation depend to a large extent on the available nutrients and on humidity. Most moulds need a rel. humidity of 80-85 % for germination. This explains the strong growth of moulds at higher air humidity [Mücke & Lemmen 1999, Sedlbaur 2001].

If in case of a building damage water and humidity, respectively, reach the insulation material and temperatures of more than 20 °C are given for a longer time, it has to be faced – according to the results of this project – a high risk of infestation with moulds on ecological material (flax, wood fibre, cork) and in parts, too, on mineralic (expanded clay, mineral wool) and synthetic materials (XPS, PU). Especially for the ecological insulation materials (flax and wood fibre) it has to be faced a change in structure as well as a negative change of the insulation characteristics.

The examination of the synthetic insulation materials at low air-humidity showed, that the germination of existing spores is possible, *if* the material is wet enough at that time. If it was possible, that a material became wet, without a rise of air-humidity for a longer time, it could probably come to a germination of possibly existing spores. The germination therefore can only last as long, as humidity is available. If a low air-humidity exists, a rapid drying will occur and the vegetation of moulds be sparse than at higher air humidity. In generally it can be considered, that for a heavy vegetation of moulds, the air humidity must be severe as well as the humidity of the material. We want to emphasise explicitly, that , as long as the material is dry, in *no* case a growth of moulds at such low air-humidity (45 %) will occur - independing of a contamination with mould-spores.

If the thermal conductivity of an insulation material rises (as it has been shown for flax), it could lead to a shift of dewpoint at the inner masonry. This in turn could lead to an infestation with moulds at the inner wall. As any infestation with moulds, this could mean a health hazard for the inhabitants, no matter where it originates from [LGA-Bericht 2001, Seifert 2002]. So it will be necessary to remove the reason for the infestation – namely the water damage.

To what extent health hazards are given by the existence of moulds on the insulation material, cannot be judged within the framework of this project. The question thereby is to what extent inhabitants are exposed to growing moulds. It should be considered that caused by an insufficient panelling of insulation material mould spores and MVOC's[2] can contaminate the indoor-air. Therefore the contaminated insulation material in any case can be considdered as a constant, hidden source of contamination. If there exist risks of health due to toxins released by moulds growing on building materials, is in generally disputed [LGA-Berich 2001, Seifert et al 2002] and will be researched at the MPA in close future.

[2] Microbial Volatile Organic Compounds – gasceous, volatile orgnic compounds that are built by microorganisms/moulds and are perceived as "bad smells" (mouldiness)

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