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Review Article

Comparison of Methods for The Examination of Biofilms in Screed Insulation Layers Made of Polystyrene with Adhesive Tape Samples, CFU and Total Cell Count

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Abstract

In the case of water damage in floor structures, especially their insulation materials, the assessment of the biomass of the affected materials is based on microscopic analysis of adhesive tape samples. Current guidelines prescribe evidence of growth structures as the main criterion for assessment. This study was used to check whether the adhesive tape analysis method is the most suitable for determining the biomass and whether the growth structures are a good indicator. It was found that examination with adhesive tape samples alone would miss many instances of damage. Even when combined with examination for colony-forming units, only a small amount of damage could be detected. In comparison, the examination of the total cell count with the acridine orange direct count was able to show a significantly higher detection rate.

Synopsis: If inappropriate analytics are selected after water damage, microbial biofilm in screed insulation layers may be overlooked and human health may be compromised. This study compares existing test methods to investigate the biofilm in the screed.

Keywords: Spores, tape samples, total cell count (TCC), Acridine Orange Direct Count (AODC), water damages, polystyrene

Introduction

Water damage in indoor spaces is a widespread and complex problem that raises monetary and organisational questions on the one hand, while on the other hand the preventive health protection of the occupants must not be disregarded. In the case of water ingress into a floor construction, the question arises as to whether microbiological contamination in the insulation materials necessitates the removal of the screed. The Federal Environment Agency's mould guide (UBA, 2017) describes the situation as a decision with far-reaching consequences. On the one hand, the health of the room occupants should be protected; on the other hand, exaggerated assessments and unnecessary deconstruction should be avoided from an indoor hygiene perspective.

In most residential buildings, a floating screed is processed with the insulating material polystyrene. For the assessment of the polystyrene, the Federal Environment Agency (UBA, 2017) recommends a microscopic examination of the biomass of the material by means of an adhesive tape sample and, if necessary, an assessment of the colony-forming units (CFU). As the cultivation of CFU takes time, this procedure is often dispensed with, and an assessment of the damage is carried out based on the results of the microscopy. The main criterion for the assessment is the detection of growth structures, e.g., hyphae. From a microbiological point of view, the question is how long these structures can be detected on a nutrient-poor material. Hyphae and mycelia decompose when no more nutrients are available to serve as nutrients for the microflora itself. This raises the question of whether this method is suitable for assessing mould damage on polystyrene.

The aim of this work is to investigate whether adhesive tape analysis is a suitable method for detecting the biomass and the microbiological growth on the insulation material polystyrene or whether other methods are better suited for this purpose.

Material and Methods

For this study, polystyrene samples from floor assemblies were randomly selected from a routine laboratory.

In the first step, a sample of adhesive film was taken from the surface of the material by pressing an approximately 6 cm long adhesive strip onto the material and pulling it off. This adhesive tape was transferred to a microscope slide, numbered, and stained with Cotton blue. All samples were analysed with a transmitted light microscope with a magnification of 1000. To obtain comparable results, 900 fields of view were counted for each sample using the 3-line method (Meider/Messal 2021) [7]. The result was extrapolated to cm^2 for comparability.

In the next step, a sample was taken from the material, weighed and, according to ISO DIN 1600-17 and 16000-21, a suspension was prepared that was needed for the next analytical steps.

In order to be able to carry out a further evaluation of the material, a serial dilution was prepared for each sample in accordance with DIN/ISO EN 16000-17 [4] and applied to the nutrient media DG 18 and malt. The dilution series was evaluated in accordance with DIN/ISO EN 16000-17 [5] after 7 days and calculated to the reference quantity CFU/gram.

The same suspension was used to determine the concentration of total cells counts (TCC) per gram by Acridine Orange Direct Count (AODC). 100µl of the sample was stained with Acridine Orange and the total cell count was evaluated by fluorescence microscopy (Meider, 2019) [8].

Subsequently, all analysis data obtained per sample were transferred to a table and evaluated. In the evaluation, separate evaluation criteria were applied for each type of analysis.

For the adhesive film sample and the CFU, the data in Table 6.2 and 6.3 of the Federal Environment Agency's mould guides were applied. German Federal Environment Agency (UBA, 2017) [10].

The samples were sorted into different categories.

The basis for the classification of the adhesive film and CFU was according to (Tables 1,2).

Subsequently, the results of all three analysis steps were compared with each other and it was investigated which analysis method best evaluates the sample and how.

Table 1: Evaluation of adhesive film samples and CFU of polystyrene from floor finishes based on mould guidelines (UBA, 2017).

Category	Specification
Background (green)	sporadic spores, no growth structures possibly CFU /g< 10^4
Contamination (yellow)	moderately many spores possibly CFU/g 10^4 - 10^5
Increased contamination (orange)	moderately many spores and growth structures possibly CFU/g 10^4 - 10^5
Proliferation state (red)	moderately many spores and growth structures possibly CFU/g >10^5 <10^6 and growth structures
Established Biomass (dark red)	many spores and growth structures possibly CFU/g >10^6

Table 2: Evaluation criteria of the total cell count" based on Trautmann/ Meider 2018.

Method	Background (green)	Contamination (orange)	Proliferation state (red)	Established Biomass (dark red)
CFU	≤ 5,0*10^3	>5,0 * 10^3 -5,0 * 10^4	>5,0 * 10^4 -5,0 * 10^5	>5,0 * 10^5
TCC	≤ 3,0*10^5	>3,0 *10^5 -1,0 * 10^6	>1,0 * 10^6 -1,0 * 10^7	>1,0 * 10^7

Theory

Correctly identifying and assessing biomass and hence microbiological damage is a complex task that can have far-reaching consequences. Deconstruction of contaminated materials is carried out under protective measures to protect workers and residents alike. On the one hand, there is a high financial factor involved, so it should only be used when necessary. On the other hand, there is a duty of care to protect the health of the residents. Studies have shown that even dried-up damage is a health hazard [3,6] and that deconstruction for preventive health protection is an appropriate measure. Another study has shown that the load on the screed insulation layer has a relevant influence on indoor air [1,2]. Further research in this area is necessary.

If it is assumed that time and drought cause the growth structures on nutrient-poor materials to disintegrate and become difficult to detect microscopically, and that the number of CFU is also greatly reduced by these factors, the difficulty of the assessment becomes clearer.

The UBA evaluation criteria state that pure microscopy of the material using adhesive film analysis is sufficient to evaluate a material. An analysis of the CFU is not necessary. It is important to note here that a sample is only evaluated as an infestation if hyphae or spore carriers are detected.

In contrast to microscopy with adhesive film analysis, which only examines the surface of the material, the analysis of the total

cell counts and CFU is an examination of the volume of the material. This means that the microorganisms in the depth of the material are also considered. For this reason, it must be noted that the results of the surface cannot be correlated with a volume examination, as was already shown in the publication by Meider / Trautmann 2018 [9].

The total cell count is a microscopic method like the adhesive film analysis, but like the CFU analysis it is based on the volume and on the same suspension. These results can therefore be related to each other [8].

Results

In the test set-up, 100 samples were analysed. For 9 samples, individual analyses could not be evaluated for various reasons. The basic population n is therefore 91 samples. 29% of the samples were in the range of the background values with all three types of analysis and are thus classified as inconspicuous. 27% of the samples showed abnormalities with all 3 methods of analysis and can thus be described as clearly conspicuous. This leaves 44% of the samples that are ambiguous, and the three methods produce different results (Figure 1).

Figure 1: Classifications of all samples with colour markings.

Tape Samples	TCC	CFU
600	420000	17000
300	230000	20000
2000	14000000	48000
500	390000	20000
400	83000000	70000
1000	4900000	11000
0	1300000	160000
100	87000	5400
327100	4700000	44000
219800	27000000	2100000
29800	7055555	3000000
1400	1600000	74
1200	2800000	180000
600	390000	120
311700	150000000	2700000
0	13000	5000
0	80000	400
300	13000	200
0	13000	350
600	21000	3000
0	11000	100
900	1200000	390
0	270000	24000
200	470000	34000
100	390000	300000
500	1600000	200000
0	200000	22000
300	1000000	110000
52200	1300000	99000
600	150000	310
900	1200000	64
1800	870000	17000
5600	3900000	250000
21600	5300000	130000
300	45000	15000
0	800000	14000
23800	4100000	140000

300	35000	560
0	41000	92
300	120000	250000
43700	5000000	260000
2100	13000000	350000
100	30000	40
1500	2000000	78
1700	6000000	8
162500	10000000	39000
0	63000	40
3300	2100000	200000
800	310000	170000
5000	2400000	130000
97100	1300000	1400000
1300	410000	190000
1700	4100000	160000
900	4000000	200000
92700	20000000	850000
33300	9700000	400000
800	720000	440000
45900	51000000	650000
200	69000	15000
9100	10000000	33000
800	300000	1900
600	500000	24000
3600	3300000	160000
500	50000	84000
0	36000	1100
300	57000	1300
200	31000	990
300	28000	180
2500	310000	6000
200	400000	72000
2900	1100000	240000
1400	450000	250000
400	210000	52000
1200	260000	44000
4200	880000	43000
2200	340000	62000
2400	290000	29000
600	120000	97000
1100	87000	62000
6600	4600000	1500000
900	150000	1700
800	89000	6100
4000	1800000	120000
0	49000	25000
1300	660000	8100
400	200000	2800

358100	25000000	290000
1800	210000	14000
900	360000	28000
700	280000	6400
900	120000	13000
500	120000	1600

Figure 1 shows the population of all samples colour-coded according to the corresponding classification. Some classifications were classified higher than the number would indicate. In these cases, additional growth structures of the moulds were detected.

Figure 2 shows all samples. This graph already shows that only a very small area of the ambiguous samples was detected with adhesive film samples - only 1% with this method alone, and 10% if other methods were also conspicuous in parallel. The total cell count alone was able to reveal a conspicuous microbiological concentration of moulds in 24% of the samples. Detection with CFU alone did not. 13% were CFU conspicuous in combination with other methods, 4% CFU and tape samples, 9% CFU and TCC.

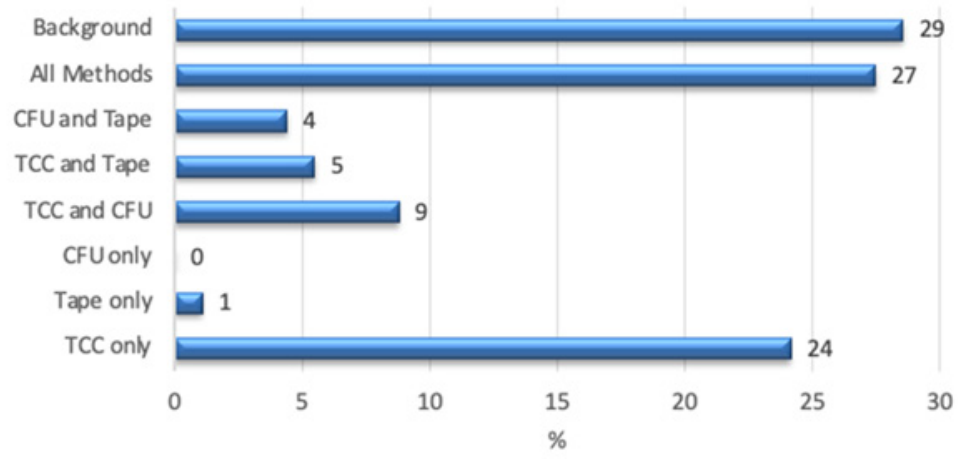

Figure 2: Detection method all samples in %

In the next figure, the inconspicuous samples have been omitted from the calculation. Here the figures become even clearer. 38% of the samples were clearly conspicuous with all three methods. In 34% of the samples, the load could only be detected with the total cell count, and in 46% the CFU was also conspicuous in addition to the TCC. Tape samples were only able to reveal a total of 16%, but only 2% on their own. CFU alone could not reveal any abnormalities, but in 13% the CFU was also conspicuous (Figure 3).

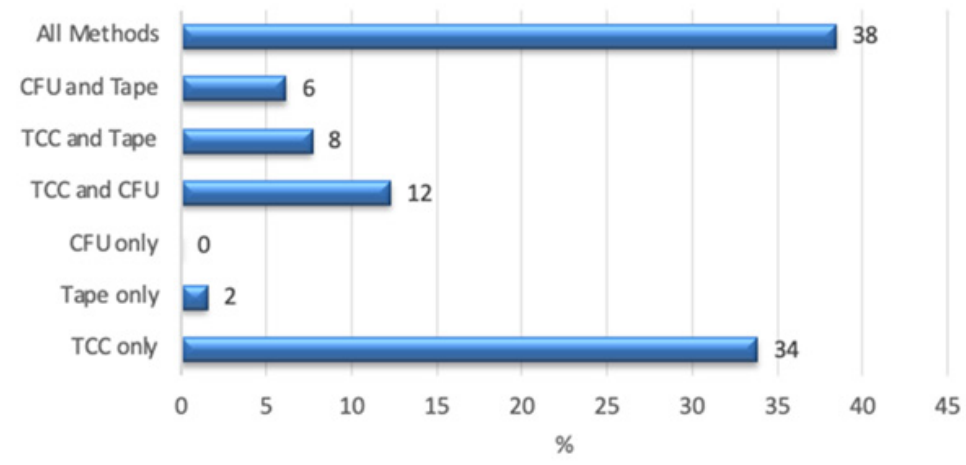

Figure 3: Detection method samples without background samples in %

In Figure 4, only the ambiguous samples were compared. More than half of the samples could only be identified with TCC. 88% of the abnormal samples were indicated with TCC. In contrast, abnormalities were diagnosed with adhesive film alone in 3% of the tape samples, but with other methods in 26% of samples. CFU alone did not reveal any abnormalities; however, in 30% the CFU were also abnormal. Ambiguous samples examined with adhesive film and CFU were found to be abnormal in 13% with both methods and 16% overall (Figure 4).

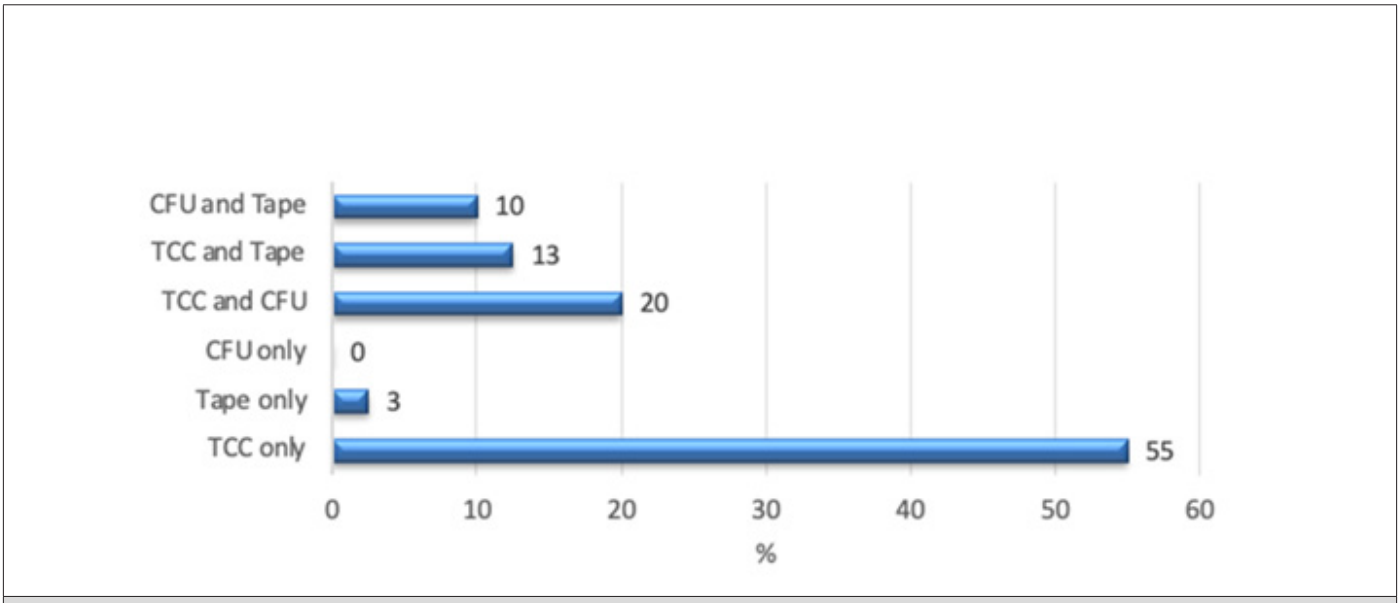

Figure 4: Detection methods without samples with background and all methods in %.

All samples were analysed in triplicate. All samples were evaluated in triplicate and the mean value applied. The total cell count showed a standard deviation of 6-8 %. The CFU showed a standard deviation of 9-15 % and the tape samples of 10-13%.

Discussion

The analysis of insulation materials from floor constructions is a main criterion for deciding whether deconstruction is necessary or not. The previous practice of using adhesive film analysis as the sole basis for decision-making is not recommended according to these data. Even in the combination with adhesive film and CFU, damage is only found in 16% of ambiguous samples. From a microbiological point of view, this can be explained by the decay of the hyphae and the reduced cultivability due to disturbing factors such as drought. This is a clear advantage in the total cell count because this method is much less influenced by disturbing factors. This is especially because the vitality of the cells has no influence in this method of analysis. Dryness, time and also most biocides do not significantly reduce the total cell counts. Even in most samples that were only detected with TCC, hyphae could no longer be detected. However, since the spore concentrations in the material do not decrease, a high microbiological load could still be detected. This is particularly important if there is no active damage when the damage is detected, but an old infestation or after a biocide application. In addition, the agreed remediation goal should also be considered. In most cases, the removal of the biomass is agreed upon and it is then important to verify this goal with a method that works independently of disturbance factors.

Conclusions

The microbiological assessment of the biomass of screed insulation layers is often carried out with adhesive film samples. The detection of growth structures is a main criterion in the assessment. However, on nutrient-poor materials such as the insulation material polystyrene, the detection of these growth criteria is difficult with adhesive film samples. This is because these structures decay after absorbing the available nutrients and are difficult to detect microscopically. The investigation shows that many instances of damage in screed insulation layers are overlooked when they are examined exclusively with adhesive film samples. Even in combination with CFU, much damage is not detected. For a reliable damage assessment, the total cell count could detect microbiological loads more reliably, as this method is less influenced by interfering factors.

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