MICROBIAL GROWTH ON CONCRETE-LIKE MATERIALS CONTAINING INDRUSTRIAL WASTES

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ABSTRACT: New recycled construction materials are launched to the markets in the boost of the circular economy and recycling targets, but not necessarily their properties of biodeterioration or microbial growth ability are not necessarily yet known exactly. Because of that, one of the project objectives was to find out how microbes, and what kind of species, grow in the surfaces of the specific concrete-like materials test pieces made in the project. At the same time, it was investigated how the different raw materials used in concrete-like materials test pieces affect the ability of microbes to grow. In this study microbes grew on the surface of concrete or concrete-like material sample test pieces (30 x 30 x 10 mm) containing green liquor dregs, blast furnace slag or Portland cement in the suitable conditions during first one month on MEA, PCA and DG18 growth medium and one more month in very poor growth conditions without medium. Two different test methods were in use. Result of both tests was the observation that microbial growth is present in all samples, regardless of the composition of the test pieces. No significant differences between the materials obtained from the factories and the recipes appeared. Method differences appeared instead. The main moulds were identified those of the genera *Penicillium*, *Fusarium*, *Paecilomyces*, *Alternaria*, *Trichoderma* and *Acremonium*. Actinomyces spp. were also in present.
Keywords: concrete, concrete-like material, green liquor dreg, microbial growth, sustainability, CO_2 reduction

1 INTRODUCTION

Environmental objectives, cleaner production targets and circular economy strive industries to utilize industrial waste more efficiently. Even with high percentage of utilization of waste in forest industry in Finland, there is still demanded to find new and better ways to increase the value of some problematic waste streams causing management costs. Partly on these reasons, JAMK has coordinated and carried out national research and development project “Sustainable bioresidual concrete” in Finland during 2018-2020. The aim of the project was to improve the environmental friendliness of concrete used in construction by utilizing pulp industry waste materials. The research was conducted in cooperation with the forest and concrete industry.

New recycled construction materials are launched to the markets in the boost of the circular economy and recycling targets, their properties are not necessarily yet known exactly. Therefore, the one of the project objectives was to find out how microbes, and what species, grow on the surfaces of the specific concrete-like materials test pieces made in the project. At the same time, it was investigated how the different raw materials used in concrete-like materials test pieces affect the ability of microbes to grow.

In general, based on in-door air research in Finland it is known that in moisture-damaged houses, moulds, yeasts and bacteria grow on the surface of concrete can be caused serious health problems. On the other hand, concrete is considered a safe building material. As Viitanen et al. (1, 2) have defined in the mould index number studies for concrete a sensitivity class of 3 or 4 depending on the age of the concrete. The index reflects the structural durability of concrete under favorable conditions for microbes. However, the durability can change, when other materials are added to traditional concrete, such as blast furnace slag, green liquor dregs or bacteria. Also content of organic compounds in material to be added has an influence to this. (3-7) It is not yet known exactly how the different, added materials affect for instance microbial growth, indoor VOC emissions, or durability of the concrete structure. (5-6, 8) The suitability of concrete as a microbial growth medium can also mean the deterioration of concrete. (9-12)

This paper focuses on the research part in the project that focused on the microbial growth in the surfaces of concrete-like material test pieces. Test materials were casted including different kind of waste materials like green liquor dregs and blast furnace slag. Traditional concrete was used as a reference.

2 MATERIALS AND METHODS

Experimental research work like production of samples and testing their properties took place in JAMK's accredited Concrete Testing Laboratory.

2.1 Materials

The dried and powdered green liquor dregs (GLD) originated from three different Scandinavian kraft pulp mills (Tab. I) and ground- granulated blast furnace slag (GGBFS) was a commercial product. Traditional concrete with Portland cement (PC) was used as a reference.

Table I: Measured pH, alkalinity and volatile organics values of GLD

GLD	pH[1]	Alkalinity[2] mmol/l, d	Volatile organics[3] w-%, d
Factory A	8 - 10	below 15	5 - 6
Factory B	10 - 11	below 50	5 - 6
Factory C	9 - 10	below 20	7 - 8

[1]SFS-EN 15933:2012
[2]Modified by SFS 3005:1987, SFS-EN ISO 9963-1:1996
[3]by SFS-EN 18122:2015 (550 °C)

2.2 Test Pieces

Concrete and concrete-like materials were prepared with different types of recipes presented in Table II. Traditional concrete include cement, water and aggregate. Concrete-like materials include also GLD or GLD and GGBFS without cement.

After casting and hardening concrete and concrete-like materials sample test pieces were cut separately for microbial test (approx. 30 x 30 x 10 mm, Fig. 1).

Figure 1: Example from test pieces before bowling and heat sterilizing.

Table II: Test pieces and their composition

Test piece	Composition	GLD Factory	Compressive strength, mean value
PM	Portland cement, filler, sand, gravel and grushed stone	no GLD	39,6 MPa (28 days)
SSFIL A	Fine aggregate/filler replaced with GLD	Factory A	33,4 MPa (28days)
SSFIL B	Fine aggregate/filler replaced with GLD	Factory B	40,8 MPa (28 days)
SSFIL C	Fine aggregate/filler replaced with GLD	Factory C	37,4 MPa (28 days)
SS20 A	Replaced 20 % share of cement with GLD	Factory A	nd.
MKSS C	GGBFS and GLD used as a binder	Factory C	38,6 MPa (90 days)
MKSS4 B	GGBFS and GLD used as a binder. (Air-entrained concrete, air content 4 %)	Factory B	26,1 MPa (90 days)
MKSS47 B	GGBFS and GLD used as a binder. (Air-entrained concrete, air content 4,7 %)	Factory B	26,9 MPa (90 days)
MKSS3 B	GGBFS and GLD used as a binder. (Air-entrained concrete, air content 4,7 %. Added accelerator 3 % by weight of the binder)	Factory B	25,64 MPa (90 days)
MKSSV A	GGBFS and GLD used as a binder. (Replaced water with silicate solution (waterglass + NaOH) to accelerate the reaction)	Factory A	39,67 MPa (14 vrk)
MKSSV B	GGBFS and GLD used as a binder. (Replaced water with silicate solution (waterglass + NaOH) to accelerate the reaction)	Factory B	65,4 MPa (28 days)

nd. = not determined

2.3 Cultivation

Air-bowled and heat-sterilized sample test pieces prepared with different recipes were exposed in the study to a mixture of microbial suspensions. Microbial samples were isolated from moisture-damaged buildings, soil and dead plant materials. Testing methods have been applied by the Healthy dwelling-guideline from the Finnish Ministry of Social Affairs and Health (13-14), as well as to various material literature (15-19).

Cultivation was carried out on three different agar growth mediums: malt extract agar (MEA), dichloran-glycerol agar (DG18) and plate count agar (PCA). There are two test methods: test 1 and test 2.

- Test 1: the sample test piece, contaminated by spreading the microbial suspension (100 µl) to top on the piece. (Fig. 2) Microbes are expected to grow on the sample test piece during the experiment.
- Test 2: the half of the growth medium, contaminated with the microbial suspension (100 µl). Microbes are expected to spread to the test pieces during the experiment. (Fig. 3)
- Test 1 and 2 from four replicate samples / test pieces of SSFIL and from two replicate samples / test piece of other in MEA and PCA medium. A few individual samples / test pieces were tested in DG18 medium by only three mould species (*Penicillium* sp, *Paecilomyces* sp. and *Aspergillus niger*), were isolated from damage building samples.

Total samples incubation time was at 25 °C for 56 days. The cultures were observed weekly, and monitored for the spread of microbes in the sample test pieces as well as the ability of the microbes to start growing on the sample piece under favorable conditions. During the first 21 days the growth were evaluated only by watching. On the 28th day of the incubation, the microbial growth was classified under light microscope and stereomicroscope. After this, the sample pieces were transferred from the culture dishes to empty Petri dishes under very poor growth

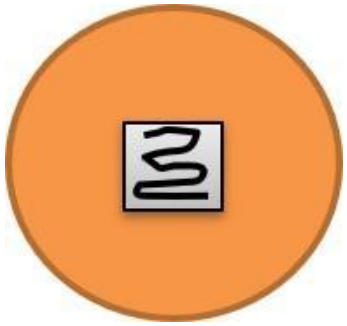

Figure 2: Experimental setup of test 1. The black line shows the microbial suspension.

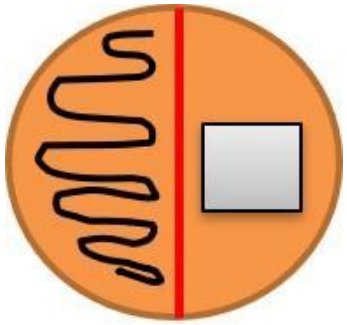

Figure 3: Experimental setup of test 2. The black line shows the microbial suspension.

conditions and incubation was continued at 25 °C next 28 days. The last detection of microbial growth was done under microscope, when experimental time had passed 56 days.

In test 1 visible microbial culture was classified into categories of no growth, low, quite a lot and rich (Fig. 4)

and respectively in test 2 into categories of no growth, in part and rich. (Fig. 5) The interpretation of categories is shown in the Table III and IV. All observations are documented.

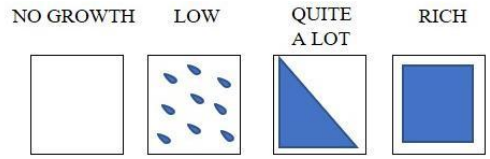

Figure 4: Microbial growth categories (blue) from the surface of the test piece during the test 1.

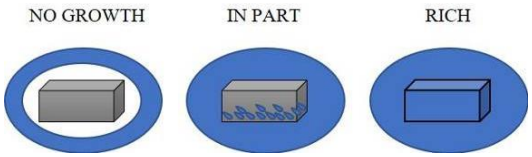

Figure 5: Categories of the spread of microbial growth (blue) to the test piece during test 2.

Table III: The interpretation of categories during test 1.

category	classified	Interpretation
no growth	0	The microbes do not start to grow in the sample.
low	1	Microbes grow poorly on the surface of the test pieces
quite a lot	2	Half of the area is covered with microbial growth
rich	3	Microbes can fully grow on the surface of the test pieces, very viable microbial growth

Table IV: The interpretation of categories during test 2.

category	classified	Interpretation
no growth	0	The microbes do not spread to the sample.
in part	1	Microbes spread to the sample and can start to grow on the edge of the test pieces
rich	2	Microbes spread fully to the surface of sample test pieces and can completely utilized the sample the growing medium, very viable microbial growth.

3 RESULTS AND DISCUSSION

Regarding the review of the results, it is particularly important to note that the observations made during the 21 days are based entirely on the classification of the microbial growth observed with the eyes. The results are presented in full, but the actual interpretation of the results is based exclusively on area classifications based on microscopes at 28 and 56 days.

After 28 days, the microbe can no longer take advantage of the favorable conditions given at the beginning of the experiment with the aid of growth media. The growth mediums dried completely in three weeks and the microbes had to survive in other ways. The aim was to wake up the microbes with growth media and then monitor their ability to utilize sample test pieces as a growth medium. Thus, it could be assumed that microbial growth is based on the microbial ability to utilize the test piece.

Only viable microbes were considered for surface area classification. If the microbial culture had dried at the end of the experiment, this area was removed from classification. However, this does not eliminate the fact that under favorable conditions these dried-up microbes are likely to start growing again.

The review of results has also taken into account that the dried and ground GLD included at least 5% volatile organic compounds. The effect of dust on microbial growth had been removed from all test pieces by air-blowing clean the surfaces of the test pieces before sterilizing the pieces. Therefore, that effect can be ruled out from the processing of the results.

The effect of alkalinity was also studied in the results and it seems, it influences amount of GLD in the test pieces. In practice, the organic content (see Tab I) also reduces or increases in the sample. However, based on the tests performed, no specific increasing effect the alkalinity and organic compounds on microbial growth was noticed.

Under microscopes was revealed that microbes can spread in the sample test pieces along the pores. Growing occurred in both, both from the surface of the test piece to the growth medium and from the growth medium to the surface of the test piece. In addition to this, on the last detection day of microbial growth onset of destruction of test pieces surface was observed, especially at the edges of the pores.

3.1 Test 1

In test 1 (Fig. 6) microbial growth was observed from the surface of the test pieces only. The results did not consider moulds or yeasts spreading on the edges of the test piece or on the surface of the MEA medium. The microbial growth classified was changed to percentage (Tab. V) and the microbial growth of the parallel test pieces was calculated averaged.

The predominant moulds observed on the surfaces of the test pieces are shown in table VI. The microbes observed are in line with previous studies. (10-12, 20-21)

Already after the first 7 days of incubation, microbial growth was visible on all growth mediums. (Fig. 7-9) In the MEA medium (Fig. 7) the surface area of the pieces containing GLD alone was somewhat more covered with moulds and yeasts than was observed on the surfaces of the test pieces containing GGBFS. In these pieces of sample, 30-90% of the surface area was covered within 28 days. In the test pieces containing GGBFS, the corresponding range was 10 to 60%. Moulds and yeasts grew the least (about 10%) in test pieces of MKSS47 B. Microbial viability either declined or remained the same under very poor growth conditions at the 56-day observation point. The microbes of test specimens of SSFIL A, SS20 A, and MKSSV continued to grow after 28 days despite the change in conditions. In these test pieces, the area coverage increased by 10 to 20%. In reference test specimens (PM), microbes covered more than 60% of the test pieces of surface area. In these sections, the microbial coverage area and microbial viability remained the same throughout the test period.

The surfaces of sample test pieces containing GLD as filler covered from 50% surface area on top of test pieces on bacterial growth medium (PCA) (Fig. 8) The variation

Figure 6: Example from mould growth (SSFIL A) on the top of surface of test pieces at the test 1 on days 28 (left) and 56 (right). Only moulds on the top of surface that are taken into account of processing the results.

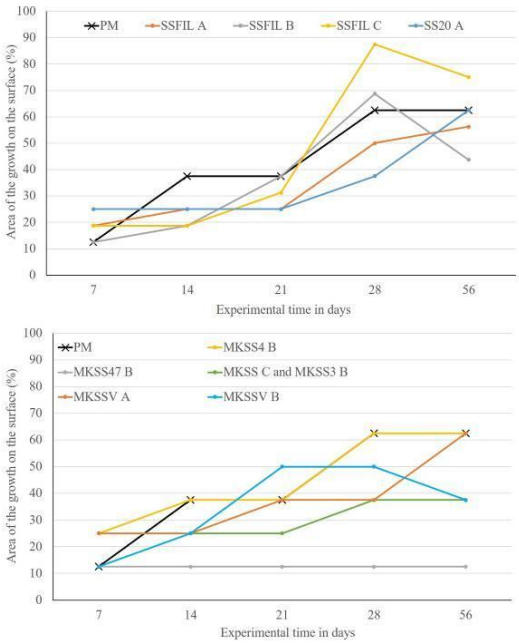

Figure 7: Mould and yeast growth on MEA medium during test 1.

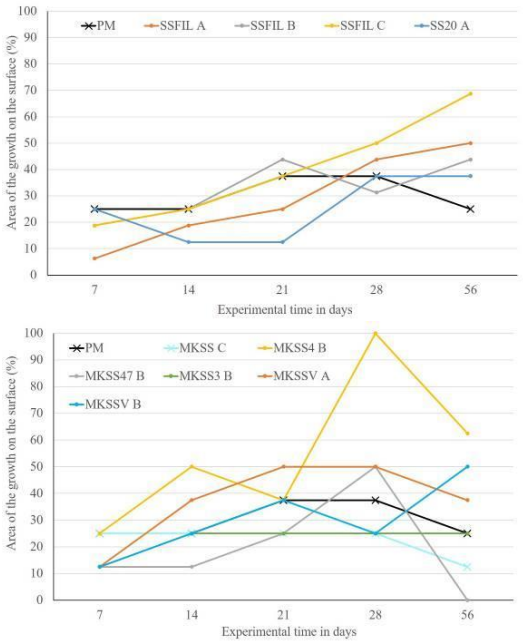

Figure 8: Bacteria growth on MEA medium during test 1.

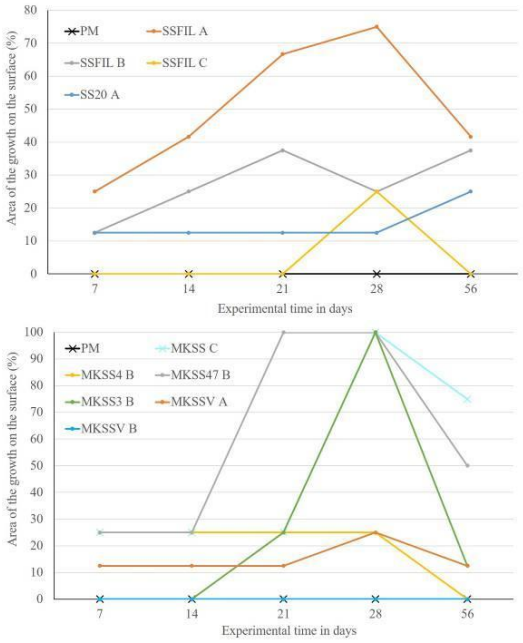

Figure 9: Mould and yeast growth on DG18 medium during test 1. Only three isolated moulds were used.

Table V: Interpretation of microbial growth classification as a percentage in test 1

Category	No growth	Low	Quite a lot	Rich
Classified	0	1	2	3
Growth	0 %	25 %	50 %	100 %

Table VI: Test 1 Predominant moulds observed from the surfaces of the test pieces. Microbes were not determinated, if surface on the test pieces were overgrow.

Test Piece	Species	
	MEA	**DG18[1]**
PM	*Trichoderma, yeast*	no growth
SSFIL A	*Alternaria Penicillium*	*Penicillium*
SSFIL B	*Fusarium yeast*	*Penicillium*
SSFIL C	*Fusarium yeast*	*Penicillium*
SS20 A	*Trichoderma yeast*	*Penicillium Paecilomyces*
MKSS C	overgrown, not specified	*Penicillium Aspergillus niger*
MKSS4 B	*Fusarium*	*Penicillium*
MKSS47 B	overgrown, not specified	*Penicillium*
MKSS3 B	*Trichoderma*	*Paecilomyces*
MKSSV A	*Acremonium Penicillium*	*Penicillium*
MKSSV B	*Trichoderma yeast*	no growth

[1]Only *Aspergillus niger, Paecilomyces* and *Penicillium* species were contaminated on the top of the test pieces.

was greater in the pieces containing. In test piece of MKSS4 B, the entire surface area had covered with bacteria, while in test pieces of MKSS C, MKSS3 B, and MKSSV B, the coverage was 25 % after 28 days. Bacterial growth increased after 28 days, especially in test pieces containing GLD as a filler. Also, in the test piece of MKSSV B, the bacteria covered the surface of the piece more than 20% more at 56 days than at the 28-day observation point. In the reference test pieces (PM), the bacteria covered about 35% (28 days) of the surface area, but after 28 days the viability reduced by about 10% in test 1 on PCA medium, the possibility of mould growth on the surface of the test pieces cannot be closed out. On the other hand, under microscopes revealed mycelium as well as spores on the surface of the test pieces, which could indicate the presence of actinomycetes. Strong odor and the absence of structures of conidium and spores typical of moulds supports this conclusion.

Penicillium sp. increased in almost all test pieces on DG18 medium. (Fig. 9) In particular, it was observed (over 70%) in test pieces of SSFIL A, MKSS C, MKSS47. *Aspergillus niger* grew only in the test piece of MKSS C together with *Penicillium* sp. *Paecilomyces* sp. was observed in both test pieces of SS20 A and MKSS3 B. *Penicillium* sp. mould was not observed in the test pieces of MKSS3. The surface area classification of these test pieces for mould was the higher.

On DG18 growth mediums, except for the test pieces of PM, SSFIL C, MKSS4 B, and MKSSV B, the moulds retained their viability throughout the study. Mainly rule, the proportion of viable moulds in the surface area decreased after 28 days. Only in the test pieces of SSFIL B and SS20 A, the surface area increased by about 10% in both samples after 28 days. The three isolated moulds in the reference test piece (PM) did not start to grow throughout the study.

3.2 Test 2

In the test 2 (Fig. 10) the spread of microbes to the edges and surface of the test piece and the ability of the microbe to survive after spreading in the materials were the focuses of the observation during the study. Because in this method on the growth mediums grew a lot of microbes, they have been left unidentified. The microbial growth of spread classified was changed to percentage (Tab. VII) and the microbial growth of the parallel test pieces was calculated averaged.

Figure 10: Example from mould growth (MKSS3 C) on the surface of test pieces at the test 2 on days 28 (left) and 56 (right). Their surface area of moulds and yeasts were monitored, which only growing on edge of the test piece.

Table VII: Interpretation of microbial growth of spread classification as a percentage in test 2

Category	No growth	in part	rich
Classified	0	1	2
Growth	0 %	50 %	100 %

As in test 1, also in test 2 was found that after the first 7 days of incubation, microbial growth was visually visible on all medium. (Fig. 11-12) On MEA medium, the microbial colonies spread rapidly to the edge or top of the test pieces. The edges of all test pieces had covered with moulds within 28 days. Test pieces of the SS20 A, MKSS4 B, and MKSSV A had 100% covered with mould after 28 days. *Trichoderma* sp. as well as moulds of the *Mucor* sp. grew strongly on MEA medium. Only in test pieces of MKSS C and MKSS3 B, the mould surface area increased by about 25% under very poor growth conditions after 28 days. In the other test pieces, the area remained unchanged or decreased at the 56-day assessment point. Significantly (approximately 50%) in test pieces of SSFIL C and MKSSV A spreading of microbes stopped after 28 days. In the reference test specimens (PM), the microbes covered more than 70% of the surface area of the test pieces. In other words, the microbes already grew on the top of test piece surface and remained viable throughout the test period.

Like mould and yeasts on PCA medium, also bacteria spread strongly to the edges and surfaces of the test pieces at the 28-day observation point. Sample test pieces, which containing GLD as a filler had completely covered the edges. In other words, at least about 50% of the total area of the piece or more had covered. In the test spread to the test piece. While, the test pieces of MKSS3 B and MKSSV B had the lowest spread (approximately 30%) and the bacteria had only partial growth on the

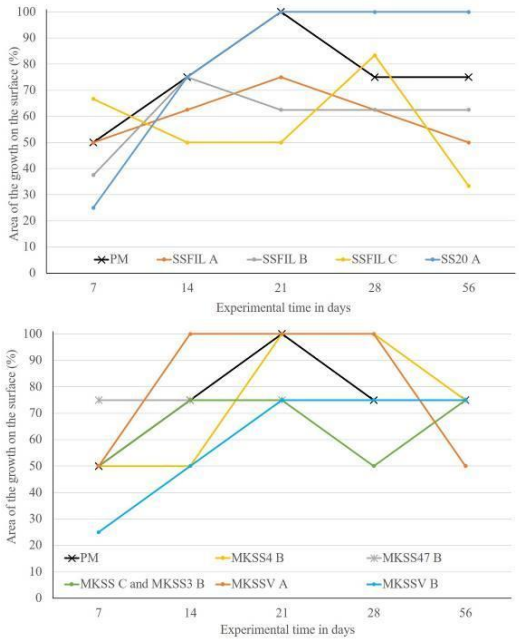

Figure 11: Mould and yeast growth on MEA medium during test 2.

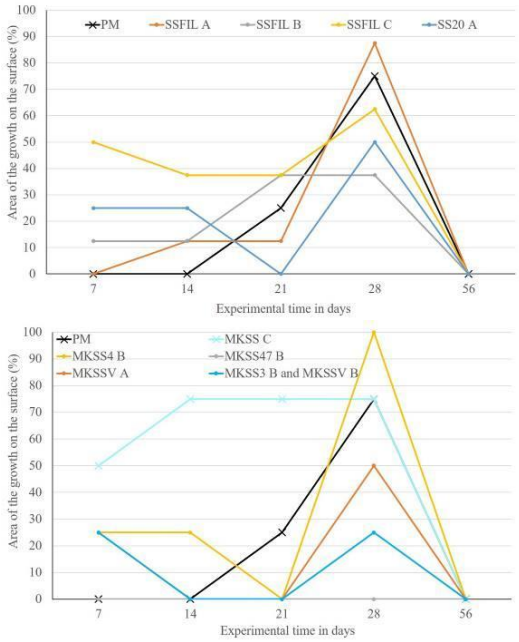

Figure 12: Bcteria growth on PCA medium during test 2.

edges of the pieces. The reference concrete test pieces (PM) covered with about 75% bacteria by observing the colonies on the top of surface of the pieces. In addition to bacteria, actinomycetes grew in all PCA plates. At the end of the test (56 days), it was found that the bacteria had dried on the surface of all test pieces under very poor growth conditions.

5 CONCLUSIONS

Based on both tests, it is evident that microbial growth is present in all samples of concrete and concrete-like material, regardless of the composition of the sample test pieces. No significant differences between the materials obtained from the factories and the recipes were found. Method differences were found instead. Test 1 was a little better than test 2, especially for moulds and yeast.

In particular, moulds can grow on the surface of test pieces and utilize the sample materials as their growth medium in both favorable and under very poor growth conditions. This was manifested an increasing in mould and yeast growth over time. Instead of, in the bacterial agar growth medium, the results were inconsistent between the tests. Bacteria grown on the test piece surface (test 1) partial increased and partial deteriorated over time, while the spread of bacteria to the test pieces (test 2) stopped completely under very poor growth conditions. There was no explanation found for the phenomenon.

The used microbial suspension contained a wide variety of moulds, yeasts and bacteria. Nevertheless, the main moulds observed were those of the genera *Penicillium*, *Fusarium*, *Paecilomyces, Alternaria, Trichoderma* and *Acremonium*. In some cases, these moulds take habitat from other species and in some cases, only one species grew over the sample test piece. *Aspergillus niger* mould was detected in only one test piece of GGBFS-GLD. In this case, the microbial mixture contained only three moulds with which the test piece had been incubated. Also Actinomyces spp. were in present in this study.

Despite the small number of replicate samples, the objectives set for the study was achieved. The methods used can detect microbial growth on concrete or concrete-like test pieces. Instead, a larger sample size is needed to compare different sample test piece recipes, as well as raw material sources so that real differences can be detected.

Finally, microscope observations also showed that microbes are able to spread along pores in test pieces or deteriorate the materials. This area was not actually the subject of the research, but it still raises the need for further research. As previous studies (6,8,10-12,15) have shown that, when new materials are introduced as a raw material for concrete, other ingredients are added to traditional concrete or used concrete-like materials as concrete, it can cause problems. In cases like this, the biodeterioration of materials and microbial growth ability in the surface of materials should be examined before the materials are approved as a concrete raw material. Especially then if the raw material is used in buildings. To this end, it would be useful to have common standard methods to use to determine such properties in practice.

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8 LOGO SPACE