TESTING THE EFFECTS BIODETERIORATION GREEN ALGAE CHLORELLA VULGARIS ON SAMPLES WITH PHOTOACTIVE COMPOSITES KAOLINE/TiO₂

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Abstract

The work deals with study of photodegradation and biodeterioration of concrete blocks with the addition of the photoactive kaoline/TiO₂ (KATI) composite. Three types of concrete blocks were evaluated: concrete with addition of the KATI (10 wt%, 20 wt%), concrete with addition of metakaoline (MK) (10 wt%) and concrete without addition of the composite. Biodeterioration tests were studied in an aqueous medium in the presence of algal culture of Chlorella vulgaris. To determine the effects of irradiation spectra, two lamps differing in spectral ranges were used to simulate light conditions similar to sunlight and artificial light with higher portion of UVB. For the evaluation of the tested blocks image analysis software “analySIS Docu” was used. Quantification of the chlorophyll amount of the algae cells was carried out according to the regulation CSN ISO 10260. Acute aquatic ecotoxicity of aqueous extracts of the material was also assessed in accordance with the CSN EN ISO 8692 regulation.

Keywords: biodeterioration, freshwater algae, aquatic toxicity, composite materials.

1. INTRODUCTION

Biodeterioration (biocorrosion) can be defined as any adverse change in material properties, which is caused by the activities of living organisms. The most important parameters that cause the growth of organisms, are: humidity, temperature, light conditions and chemical composition of the material [1, 2].

Among the organisms that cause biodeterioration are bacteria, algae, lichens, mosses, fungi (molds), higher plants and animals. Our experiment was focused on studies of green algae growth inhibition. Algae growth is influenced by light, air moisture and nutrients. They grow very well in places where water is detained (windows, cornices, balconies, etc.), on wet surfaces and in pores and crevices of stones. Algae are able to vegetate even under extremely adverse conditions [3]. Their biodeterioration effect is given by the production of metabolites (e.g. organic acids, dyes), which aggressively work with building materials and causing undesirable changes of their properties [1, 3, 4].

It pursued to slow down the course of biodeterioration process using biocidal agents because any change in the material can lead to a deterioration of its utility properties [5, 6]. Titanium dioxide shows photocatalytic properties after exposure of UV radiation, which can be demonstrated by capabilities of photodegradation of number of chemical substances. Photodegradation may be used for removal of organic pollutants, but also it can be used for growth inhibition of bacteria and algae. In this work we studied the process of biodeterioration of hardened cement paste blocks containing photoactivite composite metakaoline/TiO₂, the sample without composite and sample with metakaoline served as standard without photocatalyst for comparison of the effect of metakaoline/TiO₂ composite on grow inhibition of studied green algae.
2. EXPERIMENTAL

2.1 Tested materials

Tested samples represented hardened cement pastes: i) two sets of samples containing 10 or 20 wt.% of the photocatalytically active metakaoline/TiO$_2$ composite (KATI) developed under the project FT-TA4/025 granted by Ministry of industry and Trade of Czech Republic, where the TiO$_2$ nanoparticles are fixed on metakaolinite surface; ii) one set of samples containing 10 wt.% of metakaoline (MK) and iii) one set of reference samples presenting cement paste without any admixtures.

Prepared composites KATI61 and KATI66 were used for preparation of the testing samples (S1 and S2) of cement pastes based on the Portland cement binder. At the cement pastes containing KATI or MK admixtures, the appropriate amount of cement (CEM I 42.5R) was replaced by these materials. The obtained mixtures were mechanically homogenized and deionized water was added in the appropriate amount to obtain water to solid ratio 0.35.

The prepared mixtures were formed into the moulds (50 x 100 x 10 mm) and stored in moist environment. After 24 hours the samples were taken out of the moulds and then stored for 28 days in moist environment. After this period samples were stored for 30 days at ambient conditions. The example of the members belonging to each group of samples in the state before testing is shown in Fig. 1.

![Fig. 1 Tested materials](image)

2.2 Biodeterioration experiment

Biodeterioration studies were monitored in an aqueous aerobic environment in the presence of algal suspension. For this experiment apparatus (see Fig. 2) was designed where the autoclaved cement paste blocks were placed in. The blocks were sprinkled by nutrient medium with the green algae cells (*Chlorella vulgaris*). At regular intervals (weekly) the culture medium was supplemented.

As a source of irradiation the 18 W lamp (Juvel) that simulate daylight and 20W lamp (Repti Glo) with a higher proportion of UVB radiation were used. Once a week pH and conductivity values of the algal suspension were measured and photographs of exposed blocks were continually taken. After the expiry of the experiment duration (four weeks) the blocks were removed from the test apparatus and photographed with a digital camera. Photographs obtained were then analyzed using image analysis „Docu analysis“, where the biofilm growth was evaluated on each block.
2.3 METHODS UTILIZED

2.3.1 Image analysis „analySIS Docu“

Image analysis is nondestructive and rapid method that allows obtaining a quantitative evaluation of characteristic parameters of the examined material. Tested concrete blocks were captured using the Olympus digital camera placed on a tripod. Images were saved in JPEG format and evaluated using the image analysis.

2.3.2 Determination of the amount of chlorophyll – a

Determination of the chlorophyll – a amount was carried out according to the ISO 10260 - Water quality: Measurement of biochemical markers. Chlorophyll – a is essential photosynthetic pigment present in all green plants. Chlorophyll content in surface water is an indicator of the trophic levels. Determination of the chlorophyll concentration can provide information on the quality and potential photosynthetic activity of algae [7].

2.3.3 Algal toxicity bioassay

Evaluation of acute aquatic toxicity was performed according to the Czech Standard EN ISO 8692: Water quality - Test for inhibition of growth of freshwater green algae organism *Desmodesmus subspicatus*. The organism is commonly found in freshwater aquatic ecosystems and is therefore suitable for the aquatic toxicity screening [8]. This test was performed for the aqueous extracts prepared from samples (10 wt.% KATI10, 20 wt.% KATI10, 10 wt.% of MK and CEM) which were not used for biodeterioration tests: i) in their original solid state and ii) after their micronization using milling.

For the milled samples the extracts were prepared according to the Regulation No 294/2005 Coll. In the case of as prepared solid samples the preparation of their extracts have been followed by the Directive 89/106/EEC COUNCIL. For the determination of acute toxicity it was necessary to adjust the pH of aqueous extracts to reach the value 8.1 ± 0.3. Thus, prepared aqueous extracts were -vaccinated with organism along with control and supplemented nutrient medium. Tested samples were cultivated in a culture chamber for 72 hours under constant aeration, temperature, and lighting conditions. Every 24 hours algal growth in
suspension was assessed by using optical microscope and counting chamber. Number of cells was determined after 24, 48, and 72 h exposure [8, 9, 10].

3. RESULTS AND DISCUSSION

3.1 Quantification of biofilms overgrow using image analysis

The photographs of the samples after the 30 days exposure to algal culture with simultaneous exposure with a lamp simulating day-light conditions are shown in the Fig.3. The photographs of the samples after the 30 days exposure to algal culture with simultaneous exposure using lamp with higher portion of UVB are shown in the Fig.4.

![Fig. 3 Testing samples after 30 day exposure to algal culture, lamp simulating daylight](image)

![Fig. 4 Testing samples after 30 day exposure to algal cultures, lamp with higher proportion of UVB](image)

The results of image analysis are shown in Tab. 1. The parameter “Blocks overgrow” represents area covered by biofilm related to the total area of sample surface exposed to green algae culture.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Blocks overgrow [%] (Lamp simulating daylight)</th>
<th>Blocks overgrow [%] (Lamp with higher proportion of UVB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 wt% KATI</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>20 wt% KATI</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>10 wt% MK</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>CEM</td>
<td>57</td>
<td>32</td>
</tr>
</tbody>
</table>

3.3 Results of acute aquatic toxicity

Tab. 2 shows the test results of growth inhibition of Desmodesmus subspicatus in aqueous extracts obtained for milled samples. The results were evaluated according to the ISO 8692. Growth inhibition of green algae was proved for the extracts prepared from all of the tested materials. Sample which contained 20 wt% KATI (36%) showed the highest growth inhibition and the lowest growth inhibition was detected for the sample of pure cement paste (11%). The results of the method were comparable with the method of evaluation by the image analysis "Analysis Docu".
Tab. 2 Toxicity results of aqueous extracts to green algae *Desmodesmus subspicatus*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of cells in 1 ml after 72 hours</th>
<th>Average inhibition (stimulation) of algal growth [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Desmodesmus subspicatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 wt% KATI</td>
<td>386 250</td>
<td>Inhibition (33%)</td>
</tr>
<tr>
<td>20 wt% KATI</td>
<td>326 250</td>
<td>Inhibition (36%)</td>
</tr>
<tr>
<td>10 wt% MK</td>
<td>498 750</td>
<td>Inhibition (28%)</td>
</tr>
<tr>
<td>CEM</td>
<td>1 266 250</td>
<td>Inhibition (11%)</td>
</tr>
</tbody>
</table>

Tab. 3 presents the results of aquatic toxicity for initial solid samples. These results were evaluated in accordance with the Council Directive 89/106/EEC COUNCIL. The observed results indicate that the samples tested did not show greater inhibition than 30%, and therefore they meet the criterion set out by the Council Directive 89/106/EEC [10].

Tab. 3 Results of toxicity bioassay on green algae *Desmodesmus subspicatus* of original solid samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of cells in 1 ml after 72 hours</th>
<th>Average inhibition (stimulation) of algal growth [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Desmodesmus subspicatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 wt% KATI</td>
<td>1 156 250</td>
<td>Inhibition (16%)</td>
</tr>
<tr>
<td>20 wt% KATI</td>
<td>1 115 000</td>
<td>Inhibition (17%)</td>
</tr>
<tr>
<td>10 wt% MK</td>
<td>1 621 250</td>
<td>Inhibition (10%)</td>
</tr>
<tr>
<td>CEM</td>
<td>1 850 000</td>
<td>Inhibition (8%)</td>
</tr>
</tbody>
</table>

4. CONCLUSION

During the experiments three types of hardened cement pastes: 1. with the addition photoactivite kaoline/TiO₂ composite (10 wt% KATI, 20 wt% KATI); ii) metakaoline (10 wt% MK) and 3. without any admixture (CEM) were studied with respect to their ability to protect their surface against the green algae overgrow. The image analysis shows that the use of lamp with a higher proportion of UVB component protects green algae overgrow more effectively in comparison to experiments with lamp simulating day-light.

The aqueous extracts prepared from the milled samples show inhibition of *Desmodesmus subspicatus* growth. The largest inhibition 36% was evaluated for extracts from sample with 20 wt% KATI and the lowest inhibition were evaluated for aqueous extracts from pure cement paste (11%). Tests of acute aquatic toxicity performed for original solid samples shown the inhibition lower than 30 %.

ACKNOWLEDGMENTS

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REFERENCES