Journal of Photochemistry & Photobiology, B: Biology 160 (2016) 79–85

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Photodynamic inactivation of biofilm building microorganisms by photoactive facade paints

Annegret Preuß [a,*], Tobias Bornhütter [a], Alexander Färber [b], Christian Schaller [b], Beate Röder [a]

[a] Department of Physics, Humboldt-Universität zu Berlin, Newtonstraße 15, 12489 Berlin, Germany
[b] Sto SE & Co. KGaA, Ehrenbachstraße 1, D-79780 Stühlingen, Germany

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 7 April 2016
Accepted 7 April 2016
Available online 13 April 2016

Keywords:
Photodynamic inactivation
Singlet oxygen
Biofilm
Mold fungi
Biocides
Facade paints

ABSTRACT

This study was performed as a proof of concept for singlet oxygen generating facade paint as an alternative to conventional biocide containing facade paint for the prevention of biofilm growth on outdoor walls.
Biofilms on outdoor walls cause esthetic problems and economic damage. Therefore facade paints often contain biocides. However commercially available biocides may have a series of adverse effects on living organisms as well as harmful environmental effects. Furthermore, biocides are increasingly designed to be more effective and are environmentally persistent. Thus, an eco-friendly and non-harmful to human health alternative to conventional biocides in wall color is strongly recommended.
The well-known photosensitizer 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H,23H-porphine (TMPyP) was used as an additive in a commercially available facade paint. The generation of singlet molecular oxygen was shown using time resolved 2D measurements of the singlet oxygen luminescence. The photodynamic activity of the photosensitizer in the facade paint was demonstrated by phototoxicity tests with defined mold fungi and a mixture of microorganisms harvested from native outdoor biofilms as model organisms.
It was proven in general that it is possible to inhibit the growth of biofilm forming microorganisms growing on solid wall paint surfaces by the cationic photosensitizer TMPyP added to the facade paint using daylight conditions for illumination in 12 h light and dark cycles.

1. Introduction

1.1. Aeroterrestrial Biofilms Growing on Dry Solid Surfaces

The most common form of microorganism existence is in biofilms. Biofilms are the symbiosis of different microorganisms like bacteria, fungi, green algae, cyanobacteria, and lichen [8]; they consist of the microorganism cells and the so-called extracellular matrix (EPS). The organization in biofilms makes the organism more resistant to various environmental conditions and enables their growth, even at hostile to life places such as dry rocks and other dry and solid surfaces [3,14,19,28,39,40,43]. Growing on buildings, monuments, or technical installations biofilm forming microorganisms causes esthetic, ecological, and economical damage and additionally spore forming microorganisms like mold fungi can provoke problems to human health [15,30,32]. Therefore worldwide significant amounts of biocides are used to combat biofilm growth on solid surfaces. The release of biocides into the environment provokes new risks to human health and environmental problems [43]. Moreover, the adaptability of microorganisms caused by their fast reproduction rate generates a fast rising number of resistances to the biocides [22]. To break this vicious circle new strategies to combat biofilm forming microorganisms are necessary.

1.2. Photodynamic Inactivation an Alternative to Biocides?

Potentially the photodynamic inactivation (PDI) of microorganisms is the ecofriendly and nonhazardous biocide alternative to solve this enormous challenge mentioned above effect [1,20,23–26,34]. PDI works by a nonchemical but physical principal: The photodynamic effect; which describes the photoinduced damage of living cells by the interaction of a photosensitizer, molecular oxygen, and visible light, resulting in the generation of reactive oxygen species (ROS) in general and singlet oxygen in particular. Even though the diversity of microorganisms is huge, they have one characteristic in common: they are protected by cell walls, and the integrity of the cell walls is essential for their reproduction. Thus, the cell walls are a favorable target to combat microorganisms. Due to negative charges in the cell wall structures cationic photosensitizers are promising molecules to attack the cell walls with oxidative stress by ROS [2,12,20,33–35]. Attacking the microorganisms from outside without the necessity of an intracellular uptake of the photosensitizers [34] is the big advantage of this strategy. The idea is to immobilize the photosensitizers on the surface to create a

* Corresponding author.
E-mail address: annegret.preuss@physik.hu-berlin.de (A. Preuß).

http://dx.doi.org/10.1016/j.jphotobiol.2016.04.008
1011-1344/

photodynamically active surface without an uptake into the microbial cells. This can hopefully reduce the risk of resistance development.

Biofilms are a very complex form of life organization, especially the native outdoor biofilms containing various kinds of microorganisms. Thus the first model biofilms that have been investigated in context of growth inhibition by PDI are mono-species and poly-species bacterial biofilms of medical relevant bacteria [4]. Different materials and photosensitizers for the development of photodynamic antibacterial surfaces for medical application are also in the focus of nowadays research [6,7,27].

Besides the bacteria in outdoor biofilms green algae, cyanobacteria, and mold fungi are the difficult to combat targets. A proof of concept of the sufficient PDI of green algae [33] and cyanobacteria [10] as phototrophic primary settler in subaerial biofilms has been done already. The asexual life cycle of mold fungi especially their development of highly resistant spores [9,29] makes mold fungi very difficult to combat by biochemical strategies. The high potential of PDI to combat mold fungi and their spores has been shown by several groups [11–13,35,42].

This fortifying and successful study leads to the idea to prevent biofilm growth by oxidative stress to the cell walls using cationic photosensitizers immobilized in facade paint as a universal weapon against various subaerial biofilm forming microorganisms on outdoor walls. In this study we investigate the potential of the cationic photosensitizer 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H,23H-porphine (TMPyP) added in commercially available facade paint, natural sunlight, and atmospheric oxygen to prevent the growth of biofilms on outdoor walls.

TMPyP is well known for its ability to generate singlet oxygen under illumination. The ability to generate singlet oxygen is affected by the microenvironment of the photosensitizer molecules. Therefore the singlet oxygen generation was detected by its luminescence [16–18,36,37].

To measure the singlet oxygen luminescence on surfaces, a special setup was built which allows a two dimensional scan of the singlet oxygen generating surface.

The second question in this study is: will the singlet oxygen generated by the photoactive facade paint prevent the growth of biofilm forming microorganisms on its surface. To solve this question the phototoxicity was tested on a defined mold fungi mixtures and an undefined mixture of microorganisms harvested from native biofilms on outdoor walls. In context to the idea of using the natural sunlight the phototoxicity experiments were done with a daylight bulb emitting artificial sunlight excluding UV-light for illumination. The future application of the facade paint in natural sunlight leads to the question of the influence of nocturnal darkness on the phototoxicity. Especially the mold fungi are expected to recover in darkness by germination of their spores. Thus, the experiments are done in 12 h darkness and illumination cycles to mimic an average night and day rhythm.

The aim of the photoactive facade paint is not to inactivate existing biofilms but to prevent the formation of biofilms. Therefore, for this first study it is of minor importance to use reproducible biofilms instead of a mixture of biofilm forming microorganisms as model organisms. In summary, this is a proof of concept study to test the potential of cationic photosensitizers as an ecofriendly alternative to biocides in facade paints for the prevention of biofilm growth on outdoor walls.

2. Material and Methods

2.1. Photosensitizer

The photosensitizer 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H,23H-porphine (TMPyP) was provided by Amparo Faustino from the University of Aveiro, Portugal (Fig. 1).

2.2. Preparation of the Photoactive Facade Paints

A commercially available standard silicone faced paint (StoColor Lotusan (Art. No. 03206-064)) was used to check the concept of photodynamic inactivation of biofilm building microorganisms. For the test a Lab formulation without added in-can preservatives was used in order to exclude influence from these substances. TMPyP was incorporated by gentle stirring with a Vollrath lab mixing equipment EXF for 1 min at 1500 U/min to yield a homogeneous distribution with a dynamic viscosity of 1.950 mPa s (20 °C). As positive control an identically formulated paint was used containing a concentration of active substances of dry-film biocides in the wet paint: 600 ppm Terbutryn (Cas No. 886-50-0), 1200 ppm Isoproturon (Cas No. 34123-59-6) and 1200 ppm IPBC (Cas No. 55406-53-6).

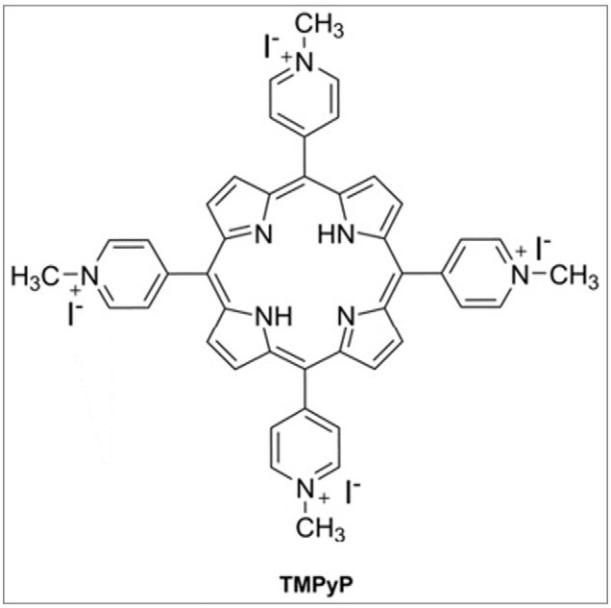

Fig. 1. Chemical structure of the photosensitizer 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H,23H-porphine (TMPyP)

Applied on filter paper circles (Schleicher & Schüll 2294 — Ref No. 342810) with a paint roller (Sto Heizkörperwalze Nylon RS8 (Art. No. 08279-005)) 2 applications with a consumption of 0.18 L/m^2 per application. 4 × 4 cm test squares were cut out from the circle filter papers. A short description of the prepared facade paint samples is given in Table 1.

2.3. Model Organisms

In this study different microorganisms were used to investigate the phototoxicity of the photoactive facade paint. One system of model organisms is a defined 1:1 mixture of *Aspergillus niger* and *Cladosporium cladosporioides* (AN/CC). Both are commercially available mold fungi provided from the *Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Culture* (DSMZ 12634, DSMZ 62121). To inoculate, the facade paint samples with the AN/CC mixture a suspension of the mold fungi conidia was used. The fungi were grown at room temperature on malt agar plates. To harvest the fungi spores, they were suspended in PBS containing 10% Tween 80. The spore suspension can be stored at −20 °C.

Table 1
Short description of the investigated facade paint samples.

Abbreviation	Short description
Ref	Negative control, facade paint without biocide or photosensitizer
$Ref^{Biocide}$	Positive control, facade paint with biocide
W^{TMPyP}	Facade paint without biocide with 0.1% TMPyP as photosensitizer