Biofilm Report: Prepared by Dr Pamela E Simpson July 2013 • Whitewater Technologies Limited

Profile

Pamela Simpson is a Chartered fellow Biologist with more than 20 years' experience in microbial control and biocide applications. Prior to founding Whitewater Technologies Limited, she worked for two major international chemical companies, where her main role was solving customers' microbiological problems by the scientific application of a wide range of speciality biocides. Her PhD was sponsored by International Paints (Courtaulds) and the compounds and techniques studied have now been developed into innovative products to replace environmentally harmful biocides. Pamela has a developed broad knowledge of the application of microbial control techniques in product preservation and antimicrobial surface protection.

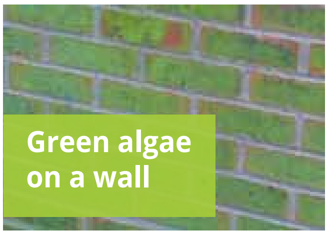
Pamela has been commissioned as an expert witness for Microbially Influenced Corrosion on pipework in a range of public buildings. She has therefore gained a lot of experience in the types of microbially species frequently associated with surface colonisation. She has experience of a range of industry sectors including oil; paper; food; paint; textiles; leather; mineral extraction and processing; adhesives; coatings and water treatment.

Colonisation of surfaces

During the recent decades there has been a general concern about the deterioration of historic buildings. Along with chemical and physical weathering factors, microbial growth plays an important role in this process. Microorganisms can colonise stone surfaces depending on stone bioreceptivity and form a microbial sub-aerial community biofilm. A biofilm forms when certain microorganisms adhere to the surface of some object in a moist environment and begin to reproduce.

Biofilm formation on surfaces usually starts with phototrophic organisms *(algae, cyanobacteria)* which use carbon dioxide (CO2) from the atmosphere and sunlight as their carbon and energy source. Heterotrophic organisms (most bacteria and all fungi) need some organic source for their growth, and this is provided by the metabolites of phototrophic organisms or by air-borne deposition. The biofilm community is therefore sometimes formed by a single microbial species, but in nature biofilms almost always consist of mixtures of many species of bacteria, as well as fungi, algae, yeasts, protozoa, and other microorganisms, along with non-living debris and corrosion products. All biofilm forming microorganisms may cause biodeterioration and degrade stone mechanically, chemically and aesthetically through the metabolic activities and biomineralisation process in these biofilms.

Studies on biofilms, along with their potential damage to a range of substrates, has led to investigations of a range of building substrates, coatings etc. It is apparent that some species, in particular pigment-producing fungi (Alternaria, Penicillium, Aspergillus), can lead to deterioration of appearance which, even after removal of the species itself, can be difficult to remove. Other species such as algae and lichens are also very brightly coloured and can rapidly develop and spoil the appearance of surfaces.


Green algae on a wall


Green algae under a microscope

Algae and Cyanobacteria

Phototrophic microorganisms (algae, cyanobacteria) can grow on stone surfaces or may penetrate the pore system of the stone itself. Phototrophic microorganisms have a direct effect on the deterioration of stone due to their pigments which cause an aesthetically detrimental effect.

Filamentous cyanobacteria *(Nostoc sp., Leptolyngbia sp., Stigonema ocellatum)* and green algae *(Demococcus olivaceus* and *Haemaotococcus pluvialis*) are capable of forming dense mucous-like layers on surfaces which produce characteristic colourations on the substrata. Nostoc sp. can produce blackish-green pigmentation which leaches into the substrate on which it is growing. The algal species produces bright green colouration of buildings as shown below:

Building before and after cleaning with AlgoClear

Biofilm Report


Red algae: collected by Jeremy Roberts and identified by Fabio Rindi, 2 May 2009.



Above left: Wall covered with fungal growth (*Alternaria sp.*).

Red algae

The genus *Trentepohlia* would not, at first glance, be taken as a green alga. Free-living species are mostly yellow to bright orange or red-brown in colour, due to the orange pigment, ***haematochrome*** (ß-carotene), which usually hides the green of the chlorophyll. The genus is terrestrial and is often found in Europe on rocks, walls and tree bark. Where they are found on buildings, they can cause severe mechanical degradation and deterioration.

Fabio Rindi and **Michael D. Guiry** (2002) have identified five species of the genera *Trentepohlia* and in urban habitats in Western Ireland: *Trentepohlia abietina* (Flotow) Hansgirg, *T. aurea* (Linnaeus) Martius, *T. iolithus* (Linnaeus) Wallroth, *T. cf. umbrina* (Kützing) Bornet, and *Printzina lagenifera* (Hildebrandt) Thompson et Wujek. These species formed perennial populations on a variety of substrata. Of the species identified, *T. aurea* and *T. iolithus* and were found on old concrete and cement walls; in particular, the latter species formed characteristic, extensive, deep-red patches on many buildings.

Western Ireland is a cold temperate area with high levels of rainfall and humidity. This area therefore supports a high level of rich and diversified algal flora. Trentepholia have been identified as one of the most common species to be isolated from walls and buildings in this region (Rindi, Guiry 2002).

Fungi

Chemoorganotrophic fungi are especially concentrated in stone crusts. They are able to penetrate into the rock material by hyphal growth and by biocorrosive activity, due to the excretion of organic acids or by the oxidation of mineral forming cations, preferably iron and manganese. This can often leave a rust-like stain on paving slabs even after washing. The deterioration activities of dematiaceous fungi also include the discolouration of stone surfaces, due to the excretion of dark pigmented melanin.

Species capable of such staining are *Alternaria, Aureobasidium, Cladosporium* and *Penicillium* spp. The photographs above right show a building wall covered with fungal growth (identified as Alternaria sp.). The microscopic images show the development of fungal hyphae and on close-up, the pigmented spores can be seen.

Filamentous fungi are composed of many cells forming thread-like hyphae which form a mycelium, or network of hyphae. Most fungal species prefer to grow at acid to neutral pH (3-6) and only require 30% moisture to survive. From these dense mats, aerial reproductive hyphae (known as conidiophores) will release millions of spores into the space directly above and hence cause massive colonisation in suitable growing areas.

The most common contaminants on substrates such as roofs, pavements and walls are the pigment producing *Aureobasidium* sp, *Cladosporium* sp, and *Alternaria* sp.

These species will rapidly spread and colonise many surfaces causing disfigurement and decay by the release of pigments and acids respectively. Indeed there are scientific studies that have identified over 22 fungal flora including filamentous micro fungi to be present on sandstone buildings. The major contaminant isolated was *Aspergillus* sp. which produced dark pigments and organic acids which were responsible for the decay of the sandstone historical monument.

The photographs below show the dark coloured species thought to be growing on sandstone paving stones.


Mosses

Botanically, mosses are non-vascular plants in the land plant division Bryophyta. They are small (a few centimeters tall) herbaceous (non-woody) plants with two main requirements; sufficient moisture and access to nutrients. They harvest sunlight to create food by photosynthesis. There are approximately 14,500 species of mosses. They are world-wide in distribution and as an opportunist, they grow on trees, pavements, garden, rooftops, and everywhere in between. Although they can occur in deserts or be submerged in water, most mosses prefer moist, shaded habitats. The moist environment of a rooftop shaded by trees is an ideal growing area for mosses. Not only does the rooftop stay

Grimmia pulvinata

Tortula muralis

Schistidium crassipilum

Schistidium crassipilum close up

perpetually moist, but nutrients are also supplemented with debris from chimneys and vegetative matter.

Mosses are best in the winter when there is plenty of water, lower light levels, and reduced temperatures. In summer mosses dry out, become dormant and take on a brown dead-like appearance.

Although mosses do not cause direct damage to roof tiles, they can cause some lifting of tiles if they grow in between tiles. As they absorb moisture, they also add weight to roofs and when they become heavy enough or dislodged by bird activity etc, they can fall in gutters and down pipes and cause blockages.

The most common species of mosses in towns and cities in the UK are the grey-cushioned grimmia, *Grimmia pulvinata;* thickpoint grimmia, *Schistidium crassipilum;* and wall screwmoss, *Tortula muralis.*

Lichens

A lichen consists of 2 or more partners that live together symbiotically, with both of them benefiting from the alliance. One partner is a fungus. The other is either an alga (usually a green alga) or a cyanobacterium, which is sometimes called a blue-green alga although it is more closely related to bacteria than algae.

The bluegreen algae occur as symbionts in about 8% of known lichens. The most commonly occurring genus is Nostoc. The majority of the lichens contain either Chlorophyta (green algae) or Xanthophyta (yellow-green algae). About 90% of all known lichens have a green alga as a symbiont, and among these, Trebouxia is the most common genus, occurring in about 40% of all lichens. The second most commonly represented green alga genus is *Trentepohlia.* Overall, about 100 species are known to occur as autotrophs in lichens.

The alga or cyanobacterium is able to use sunlight to produce essential nutrients by photosynthesis that feed both partners. The fungus creates a body, called a thallus, in which they both live. The fungus also produces chemical compounds that may act as sunscreen to protect the photosynthetic partner.

There are more than 1,700 species of lichen in Britain. Approximately 30,000 species of lichen have been described and identified worldwide. The algal partners in lichens can be found living on their own in nature, as free-living species in their own right. The fungal partners in British lichens are recognisable Ascomycetes or Basidiomycetes. However, they have come to need the right kind of algal partner in order to survive. Unlike other fungi or indeed their algal partner, they cannot survive on their own.

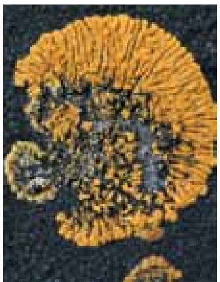
Lichens have a variety of different growth forms. The simplest lichens are crusts of loosely mixed fungal hyphae and algae. Others are more complex, with leafy or shrubby forms like miniature trees, also having specialised structures to attach them to a surface.

Crustose (or Crustaceous) lichens are, as their name suggests, encrusting forms which spread over and into the surface of their habitat. They cannot be removed from the surface without crumbling away.

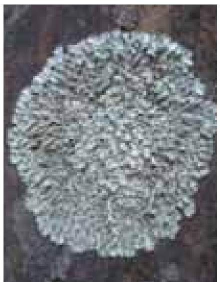
Foliose lichens are lichens with leafy lobes, which spread out in a horizontal layer over the surface. They are attached by root-like threads and can be easily removed with a knife.

Fruticose lichens are shrubby forms with many branches. They can be removed from the surface by hand.

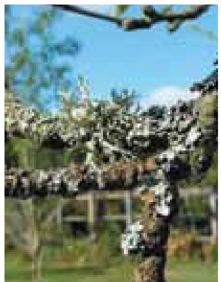
A major advantage of lichens is that they are poikilohydric, meaning that though they have little control over the status of their hydration, they can tolerate irregular and extended periods of severe desiccation. Like some mosses, liverworts and ferns, upon desiccation, lichens enter a metabolic suspension or stasis which the cells of the lichen symbionts are dehydrated to a degree that halts most biochemical activity. In this state, lichens can survive wider extremes of temperature, radiation and drought. Hence this is one reason why these species can be found on substrates not normally able to be cultivated by plants.



Crustose encrusting lichens (Calaplaca sp.)



Foliose leafy lichens (Lichina sp.)



Fruticose shrubby lichens

Treatment of surfaces with a biocidal wash

AlgoClear is a QAC which is effective at all the target organisms usually associated with surface spoilage. In the case of the lichens and mosses, it is the algal component of the consortium that is to be targeted and as such with destabilise the lichen or moss and cause them to dye. Hence Algoclear is acting as a remedial treatment of surfaces to reduce the presence of microbial species present which are causing discolouration or destruction of the surface. QAC's are extremely effective at killing a range of species frequently seen on substrates. This concentration is usually within the range 0.5-1% QAC but is very dependent upon the conditions in which it is being used, and the longevity of inhibition required.

Below are some examples of the effectiveness of treatment by **AlgoClear:**

1: Algae removal from roofing tiles

Trentepholia spp. produce extensive pink or red growth and cause major discolouration of painted buildings. The picture above is an example of such discolouration on painted and pebble dashed building walls in Ireland. Treatment of these walls with **AlgoClear** will remove the disfiguring algae as highlighted above.

2: Fungal removal from sandstone paving stones

3: Moss removal from roof tiles

Whenever a surface is to be cleaned, it is important to use a biocidal agent to kill the species present but it is important to do this when the weather is appropriate. For example, biocides/cleaners may be washed out by rain before they have had time to act. Biocidal treatments should therefore be undertaken during dry conditions. Windy weather may lead to excessive drift of biocidal spray and pose health and environmental hazards. The solution of an appropriately chosen biocide must then be carefully prepared and applied in strict accordance with the manufacturer's recommendations for safety and protection of operator and the environment.

Worldwide spraying and brushing of diluted biocidal solutions are the best methods of application. Brushing is recommended when the hard surface is in fairly good condition and the area required to be treated is relatively small. Spraying is the preferred choice for deteriorated hard surfaces and larger areas such as roofs and walls.

When handling biocides, always wear rubber gloves, safety glasses and mask or a visor.

Use biocides safely. Always read the label and product information before use.

AlgoClear SOFTCLEAN

UK: TVSP Ltd, PO Box 1945, Maidenhead, Berkshire SL6 2DD
Tel: (44) 1628 687 022 | www.algoclear.com | info@algoclear.com | Contact: Francois Moal
IRELAND: Cropcare Ltd, Willowgrove, Delgany, A63 XC97, Co. Wicklow, Ireland
Tel: (353) 1287 4485 | www.softwashireland.ie | Contact: john@cropcare.ie