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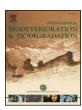
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## Monitoring microbial soiling in photovoltaic systems: A qPCR-based approach

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#### ABSTRACT

Soiling of photovoltaic (PV) systems compromises their performance causing a significant power loss and demanding periodical cleaning actions. This phenomenon raises great concerns in the solar energy field, thus leading to notable research efforts over the last decades. Soiling is caused by a dual action of dust deposition and biofouling. However, surprisingly, the microbiological contribution to PV soiling is often overlooked or underestimated. In this study, a variety of qPCR-based methods have been developed to quantify the microbial load of fungi, bacteria and phototrophs on PV panels. These protocols were evaluated by comparison with culture-dependent methods, and were implemented with real solar plants for two years. The results show that the developed molecular methods are highly sensitive and reliable to monitor the microbial component of the soiling. Fungal biomass was clearly dominant in all analysed PV modules, while bacteria and phototrophs showed much lower abundance. Light microscopy and qPCR results revealed that melanised microcolonial fungi and phototrophs are the main biofilm-forming microorganisms on the studied solar panels. In particular, the fungal qPCR protocol is proposed as a useful tool for monitoring of PV soiling, and investigating the microbial contribution to specific soiling cases.

#### 1. Introduction

In the last decades, there has been a great development of renewable energies, more specifically of solar energy including concentrating solar-thermal and photovoltaic (PV) technologies (Jacobson and Delucchi, 2011). The latter have particularly seen significant expansion in terrestrial applications during the last decade.

The productivity of PV systems depends mostly on climatic conditions and solar irradiance, but also on soiling, a complex phenomenon that can basically be defined as the accumulation of particles on the surface of PV modules, regardless of their origin. This process compromises the performance of PV systems causing important power losses, and demands periodical cleaning with the corresponding increase in expenses (Maghami et al., 2016; Costa et al., 2016). The term "soiling" is habitually used to describe the accumulation of dust, dirt, snow, plant debris, pollen and bird droppings on solar panels (Maghami et al., 2016). Surprisingly, despite the well-known prevalence of microorganisms in all environments, including dust and plant debris, their

contribution to this phenomenon is overlooked or underestimated. To illustrate this fact, it is noteworthy to mention that just one (Shirakawa et al., 2015) of the 198 dust/soiling PV papers published from 2013 to 2015, compiled by Costa et al. (2016), was focused on soiling-causing microorganisms.

To the best of our knowledge, a total of four studies that are somehow related to microorganisms and biofilm development on PV panels have been published to date. Noack-Schönmann et al. (2014) initially pointed out that sub-aerial biofilms (SAB) growing on solar panels, like those from buildings and rocks, are mainly composed of phototrophs and melanised microcolonial fungi (MCF), which absorb and scatter the incident light. Laboratory experiments with model and natural biofilms on glass demonstrated that they block up to 70% of light transmission. Melanised fungi, pigmented bacteria as well as diverse phototrophs were the dominant microorganisms on solar panels in the moderate climate (Noack-Schönmann et al., 2014) as well tropical environments (Shirakawa et al., 2015). Considerable biofouling on PV facilities in tropical environments proceeds very fast. Shirakawa

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et al. (2015) reported that PV modules from Sao Paulo (Brazil) displayed significant power reductions (7% after 6 or 12 months and 11% after 18 months), which were attributed to the dual nature of soiling by both particle deposition and SAB fouling. An additional study (Shirakawa et al., 2016), exposing different modern glasses for 5 months to the same environment, reported similar colonizer microorganisms (fungi and phototrophs) and different bioreceptivity of glasses. TiO2 coating (self-cleaning glass) inhibited the formation of fungal hyphae, thus reducing the overall biofilm cover. In contrast, silver nanoparticle polymeric coating was not effective in reducing SAB formation. In addition, Dorado-Morales et al. (2016) recently characterised the microbial communities dwelling on solar panels in a Mediterranean city (Valencia, Spain) by using high-throughput amplicon sequencing. The studied PV modules harboured a highly diverse microbial community with more than 500 different species per panel, most of which belong to drought-, heat- and radiation-adapted bacterial genera, and sun irradiation-adapted epiphytic fungi.

In addition to the PV power loss due to microbial soiling, SAB development on the surface of PV modules has the potential to cause secondary problems in the underlying glasses, such as corrosion and biodeterioration. These deteriorative processes are well-known in historical and optical glasses (Drewello and Weissmann, 1997), being especially relevant in medieval glasses of church windows (Piñar et al., 2013). The typical biodeterioration symptoms – etching, pit corrosion, and leaching – were successfully reproduced in laboratory experiments using similar glasses incubated with microorganisms for 6 months (Gorbushina and Palinska, 1999; Rodrigues et al., 2014). However, considering the different properties of modern glasses used in PV modules, as well as their relatively short operating life (20–25 years), the expected impact of such deterioration processes on PV systems should be rather low.

Considering the lack of knowledge about the biological contribution to soiling processes, sensitive and reliable methods should be developed to quantify the microbial load on PV systems. Such quantitative approaches would be useful tools to monitor soiling of PV modules and design appropriate cleaning strategies, as well as to investigate the role of microorganisms and biofilms on soiling problems.

Molecular biological techniques possess key advantages as quantification tools, such as independence of cultivation, simplicity, speed, specificity and sensitivity. In this sense, the real-time quantitative polymerase chain reaction (qPCR) is considered the key technique for nucleic acid quantification, and quantifying DNA by qPCR has been used to determine biomass equivalents. Many qPCR-based protocols have been developed for diagnostics and microbial quantification in medicine, agriculture and environmental sciences (Zhang and Fang, 2006; Biassoni and Raso, 2014), including studies focused on material biodeterioration (Ettenauer et al., 2014; Martin-Sanchez et al., 2016, 2018). However, to the best of our knowledge, there is no reported study using qPCR on neither glass material nor PV modules to date.

The goals of this study are (i) to develop and evaluate a variety of qPCR-based methods to quantify the microbial load on PV panels, and (ii) to compare them to culture-dependent methods. Following this qPCR approach, three classes of microbial targets with relevant ecological roles (fungi, bacteria and phototrophs) have been monitored in real PV facilities to assess the dependence on inclination, orientation and type of PV modules.

#### 2. Materials and methods

#### 2.1. Samples from photovoltaic facilities

In October 2015, for preliminary evaluation of sampling protocol, two PV modules at the Faculty of Physics from the Freie Universität Berlin (FU) (Fig. 1a) were sampled, with GPS coordinates  $52^{\circ}$  27′ 24.4″ N,  $13^{\circ}$  17′ 41.36″ E. This PV facility, composed of thin-film cadmium telluride (CdTe) modules, was installed in 2008. A total of eight

samples, containing all material (including dirt, debris and biofilms) present on the study areas, were collected. Two surface areas,  $25\,\mathrm{cm}^2$  ( $5\times5\,\mathrm{cm}^2$ ; Fig. 1b) and  $100\,\mathrm{cm}^2$  ( $10\times10\,\mathrm{cm}^2$ ), were analysed in two zones of the modules (top and bottom) showing different degrees of contamination (Fig. 1a).

Afterwards, for two years (Oct/Nov 2015-16), sixteen different arrays, each composed of 36-40 PV modules with the same inclination, orientation and type of module, were sampled in triplicate from the Karlsruhe Institute of Technology (KIT), Karlsruhe (Fig. 1c), GPS coordinates 49° 5′ 57.79" N, 8° 26′ 16.004" E. This 1 MW solar power storage park contains 102 different arrays that were installed in June 2014. It is important to note that there was no cleaning maintenance during the time of this study. Each replicate sample was collected from the surface corresponding to one PV cell (225 cm<sup>2</sup>) located at three different zones of the array, in the row of cells second from the bottom (Fig. 1d-e). The lowest rows of PV cells, where water and particles were accumulated and in some arrays green biofilms were evident, were discarded because they were not representative of the general contamination degree of the arrays. More details about the studied arrays, such as inclination, azimuth direction and type of module, are shown with the results (Table 1).

Four additional samples, two per each photovoltaic facility, were collected in November 2016 to characterize biofilm morphology using a Zeiss Axio Imager 2 light microscope.

In total, 60 biofilm samples, 10 from FU and 50 from KIT, were analysed in this study. They were collected in 15 ml Falcon tubes containing 5 ml PBS buffer with 0.04% Tween 80 using CleanTips Swabs (TW Texwipe, USA). Samples were stored at 4 °C until the subsequent analyses, microscopy, culturing of microorganisms, and DNA extraction for molecular quantification by qPCR.

#### 2.2. Counts of cultivable microorganisms

Total viable counts of bacteria and fungi were determined for KIT samples collected in 2015. For that purpose, PV samples were homogenised by vigorous shaking in a vortex for 10 min, and ten-fold diluted four times.  $100\,\mu l$  aliquots of original suspensions and their corresponding dilutions were plated in triplicate on diluted tryptone soya agar (DTSAcy;  $4\,g\,l^{-1}$  TSA (Oxoid, England),  $15\,g\,l^{-1}$  agar and  $25\,mg\,l^{-1}$  cycloheximide) for bacteria, and diluted malt extract agar (DMEAc;  $1\,g\,l^{-1}$  malt extract,  $1\,g\,l^{-1}$  peptone,  $1\,g\,l^{-1}$  glucose,  $20\,g\,l^{-1}$  agar and  $50\,mg\,l^{-1}$  chloramphenicol) for fungi. Culture plates were incubated at  $25\,^{\circ}\text{C}$  in the dark and the colony forming units (cfu) for both bacteria and fungi were counted after four days.

#### 2.3. Isolation and identification of reference strains

After determination of the total viable counts, and based on the morphology of grown colonies, some of the most abundant cultivable bacteria and fungi were isolated in pure cultures on the same media to be used as reference strains in qPCR analyses. Bacterial suspensions in 30% glycerol were stored at  $-80\,^{\circ}\text{C}$ , and fungal strains were grown on MEA slants and stored at  $4\,^{\circ}\text{C}$  until subsequent analyses.

Molecular identification of isolated strains was performed by PCR and sequencing of ribosomal markers, 16S rRNA gene (16S) for bacteria, and rDNA internal transcribed spacers (ITS) for fungi. Genomic DNA extraction, primers and PCR conditions, as well as Sanger sequencing, were performed as previously described by Martin-Sanchez et al. (2018). Obtained sequences were submitted to the European Nucleotide Archive (ENA, EMBL-EBI) under the accession numbers LT797544- LT797551. Identification of strains was based on comparison of their rDNA sequences with GenBank using the BLASTn algorithm from EzBioCloud's Identify Service for bacteria or the National Center for Biotechnology Information (NCBI) for fungi.

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