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Monoglyceride to protect building materials against microbial proliferation

T. Verdier^{1,a}, A. Bertron¹, R. Valentin², G. Nyame Mendendy Boussambe², Z. Mouloungui² and C. Roques³

Abstract – Use of bio-based products from the recycling of glycerol, a valuable by-product of agro-industry as antimicrobial coatings may be an eco-friendly alternative to the classic ways of protecting building materials against microbial proliferation such as metal-based treatments, biocides, etc. Monoglycerides (MG) can be synthesized starting from oleochemical synthons such as glycerol and fatty acids. Numerous studies have shown the antimicrobial efficiency of fatty acids and esters against various microorganisms. This paper focuses on evaluating the antimicrobial potential of a specific MG molecule with the aim of incorporating it in a semi-transparent aqueous coating intended for building materials. Three types of experiments were carried out: (i) evaluation of bactericidal activity in liquid phase, (ii) evaluation of antibacterial activity according to JIS Z 2801, and (iii) evaluation of the resistance of coated building materials to natural microbial contamination. Results showed that the MG molecule tested possessed strong antibacterial properties. These promising results highlight potential of such molecules in the protection of building materials and encourage further studies.

Key words: Monoglyceride / coating / antimicrobial / microbial proliferation / building materials

1 Introduction

Building materials in indoor and outdoor environments are exposed to microorganisms (mainly fungi, algae, and bacteria) daily and, in many contexts, promote conditions for their proliferation, e.g. by providing moisture and nutrients. Numerous man-made structures, including sewers and pipes [1], moisture-damaged buildings [2–4], historical monuments [5], etc. have been reported to be heavily contaminated by microorganisms. Microbial growth on building materials may have detrimental consequences by inducing physical and chemical changes. Their proliferation can lead to biodeterioration, causing aesthetic and structural damage to the concerned buildings. For example, their stability can be affected by the penetration of fungal hyphae or by carbonates dissolution and the solubilization of some elements [5].

In addition, proliferation of microorganisms in indoor environments is of growing concern because they can degrade indoor air quality and consequently impact the

health of building occupants [6, 7]. Their contribution to the Sick Building Syndrome is widely reported in the literature. Microorganisms are present in indoor air and may proliferate on indoor surfaces when relative humidity of material is sufficient. The microorganisms growing on indoor building materials may release aerial contaminants (bioaerosols) [8, 9] that can be harmful to people exposed to them.

Given these issues, it has become important to find ways to protect materials against microbial proliferation. In the past two decades, there have been many attempts to develop antimicrobial protections for building materials. The classic solutions studied include biocides [10, 11], metal-based (typically Ag-, or Cu-bearing) treatments [12, 13] and photocatalysis (TiO₂, ZnO, etc.) [14–16]. Apart from their cost, the potential disadvantages of some of these solutions are the risk of release into surroundings, leading to a potential hazard for human health and ecosystems. Thus, developing new solutions that are less expensive and more respectful of the

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environment is a challenge for current research in field. Bio-based substances with natural antimicrobial properties, such as glycerol esters, would be an interesting solution. Glycerol is massively produced in agro-industry [17, 18] and fatty acid glycerol esters present specific intrinsic properties towards interfaces which have favored its use in various fields, including cosmetic, food, medicine, textile, etc. Monoglycerides (MG) are molecules composed of a fatty acid linked to a glycerol molecule by an ester bond. Antimicrobial properties of such molecules have been under study for a few years now. They have been shown to inactivate a wide range of microorganisms, including bacteria, yeasts, fungi, and viruses [19]. The incorporation of such molecules in coatings or in mass of building materials against microbial proliferation would be a low cost eco-friendly solution.

The objective of the present study was to carry out several tests to evaluate the antimicrobial potential of a specific MG molecule. Three types of experiments were carried out: (i) evaluation of bactericidal activity in liquid phase, (ii) evaluation of antibacterial activity according to JIS Z 2801, and (iii) evaluation of the resistance to natural microbial contamination of coated gypsum board.

2 Materials and methods

2.1 Chemical materials

The monoglyceride (MG) used in this study were synthesized and physicochemically characterized following methods described in our previous study [20]. The molecules are composed of 11-carbon chains length but the exact chemical formula is confidential.

The monoglyceride molecules were in the form of an aqueous and viscous liquid of light brown color. In the different experiments of this study, the product was diluted in distilled water with stirring. In the paper, this final product is referred as MG or MG-coating.

2.2 Biological material

Escherichia coli CIP 53126 was obtained from Institute Pasteur Collection, Paris, France. The strain was preserved at $-80\text{ }^{\circ}\text{C}$ in Eugon medium supplemented with 10% glycerol. Bacterial cells were pre-cultured on a slant nutrient agar in tube. Then two subcultures on Trypcase Soy Agar (TSA) in petri dishes were incubated at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 16 to 24 h before each experiment.

2.3 Evaluation of bactericidal activity in liquid phase

The test procedure is those described in European standard NF EN 1040 [21]. The experiment is based on the contact between a chemical product and a bacterial suspension in a test tube. The tube is placed in a water bath to control temperature for a relatively short period

of time (5 and 15 min). Then, the chemical agent is either neutralized with a specific neutralizer or separated from bacterial cells by a filtration step (NF EN 1040 [21]). In our experiment, the filtration approach was chosen. Then, residual viable bacteria after contact with a chemical agent were numerated (CFU – Colony Forming Units) on membrane (= assay). The objective was to determine the bactericidal power of the chemical agent.

For each trial, one plastic loop of the last bacterial subculture was dispersed in sterile distilled water and the concentration was adjusted to between 1.5×10^8 and 5.0×10^8 CFU/ml with a spectrophotometer (concentration was assessed by CFU-counting on TSA plates incubated for 48 h at $36\text{ }^{\circ}\text{C}$). A solution of MG diluted in sterile distilled water was added to the suspension so that the final concentrations were 20% MG by mass and between 1.5×10^7 and 5.0×10^7 CFU/ml. After 5 and 15 min, the mixture was diluted and filtrated on Isopore membranes (Millipore). Membranes were deposited on TSA plates and incubated at $36\text{ }^{\circ}\text{C}$ for 24 h to 48 h. The number of viable cells was estimated in CFU/ml As specified in standard [21], control validations were carried out in parallel in order to verify the absence of lethal conditions during the experiment and to validate the filtration procedure (separation of chemical agent and bacterial cells).

Four trials were conducted with 2 periods of contact (5 and 15 min). The final reduction rate was calculated as the difference between the number of bacteria before contact and the number of bacteria after contact:

$$R = \log \left(\frac{N_0}{N_a} \right)$$

where R is the reduction rate; N is the number of CFU at the beginning of the experiment; N_a is the number of CFU from the test suspension after contact (counted on membranes).

2.4 Evaluation of antibacterial activity according to the standard JIS Z 2801

This test was carried out in order to evaluate the antibacterial activity of MG as a coating for supports. The overall test procedure is described in standard JIS Z2801 [22]. It is based on contact between a drop of a bacterial suspension and an antibacterial surface under specific conditions. After contact, bacteria are recovered with a specific surfactant, diluted and each dilution is dispersed in Petri dishes with TSA. Dishes are then incubated for 48 h to determine the number of CFU. Antibacterial activity is then calculated as the difference between the number of viable bacteria after contact with the tested surface and the number of viable bacteria after contact with a control surface (without antibacterial properties).

In our experiment, Isopore membranes (Millipore) were coated with 0.5 ml of a solution of MG in distilled water. Two concentrations were tested: 5% and 1%. Coated membranes were then laid in Petri dishes and

placed under a sterile flow hood for air drying. After drying, they were inoculated with a bacterial suspension adjusted around 2.5×10^5 CFU/ml. Membranes without coating were also inoculated as control samples. All samples were then placed in an airtight container and incubated at 36 °C and 90% RH for 24 h. The relative humidity was maintained with a saturated solution of KNO_3 . After 24 h, bacteria were recovered with the solution required in the standard, inoculated in TSA plates, and incubated for 48 h, as described in the standard [22]. Samples were tested in triplicate. The antibacterial activity was calculated as follows:

$$A = \log \left(\frac{N_C}{N_{MG}} \right)$$

where A is the antibacterial activity, N_c is the number of bacteria (CFU) from control samples and N_{MG} is the number of bacteria (CFU) from MG samples.

2.5 Resistance to natural microbial contamination on building materials

The purpose of this test was to evaluate the capacity of MG-coating to protect construction materials from microbial growth.

Commercial water repellent plasterboards (13 mm) were cut into samples of 5×10 cm. A 20% aqueous solution of MG was sprayed onto test samples which were left to dry in ambient air. Samples not sprayed with MG were used as controls. All samples were stored without protection in a closed, moist chamber at 100% RH and ambient temperature (≈ 20 °C) for at least 8 weeks. They were then scanned in order to quantify the natural colonization of surfaces as described in previous studies [23, 24].

The recovery rates were evaluated by image analysis using Sage's *ImageJ* plugin [25] for K-mean clustering.

3 Results and discussion

3.1 Bactericidal power of MG in liquid phase

The bactericidal rates of MG's suspension (20% by mass) are 1.81 ± 0.37 log ($p < 0.01$) after 5 min and 3.81 ± 0.34 log ($p < 0.001$) after 15 min of contact (Fig. 1). These values are lower than basic bactericidal activities of the chemical disinfectants usually tested according to standard EN 1040, which are about 5 log after 5 min of contact. Nevertheless, the bactericidal activities observed in this test are significant ($p < 0.001$) and quite important for the aim of this study, i.e. protecting building materials against microbial proliferation. These values are close to those from the work of Bergsson et al. [26, 27], which observed inactivation rates of bacteria (*Helicobacter pylori* and *Streptococcus aureus*) from 2 log to 8 log in 10 min of contact, depending on the monoglyceride and its concentration.

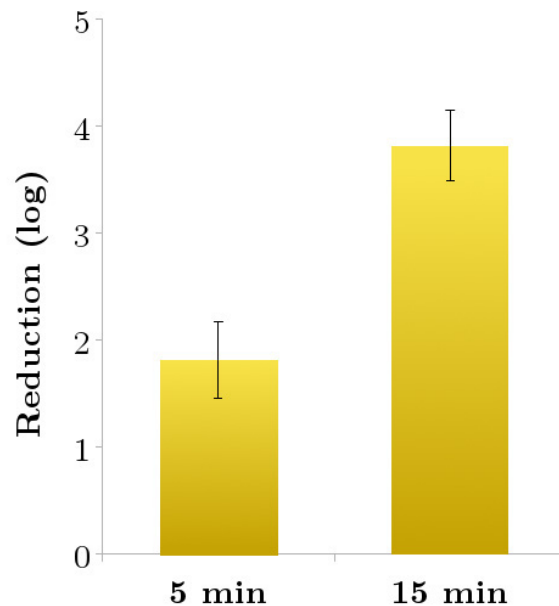


Fig. 1. Bactericidal activity (NF EN 1040) of the MG on *E. coli* CIP 53126. Mean \pm s.e., $n = 4$.

For information, the observed inactivation rates are close to those of irradiated TiO_2 in optimal laboratory conditions, i.e. 1 g/l in aqueous solution, UV-irradiated at 550 W/m^2 [28], and are higher than those of TiO_2 irradiated with low intensity UV radiation (5 W/m^2) [16]. Thus, monoglycerides showed a bactericidal power at least as high as those from the classic studied solutions building materials protection against microbial proliferation.

The mechanisms of action of monoglycerides are still unknown but they are likely to take place at cell membrane [26, 29, 30]. In their work on Gram-positive cocci, Bergsson et al. [26] suggested that MG molecules penetrated through the peptidoglycan layer without causing significant damage. Regarding cytoplasmic membrane, many studies on fatty acids have reported interactions with the electron transport chain, impacting oxidative phosphorylation (metabolic pathway in the production of energy) or with other proteins that are involved in the transport of certain compounds within the cell [31–33]. However, these studies worked on fatty acids only and results could be different with monoglycerides. Desbois and Smith [19] listed other processes related to the action of fatty acids and their esters that contribute to the death of bacteria or the inhibition of their growth: inhibition of enzyme activity, modification of nutrient absorption, generation of toxic peroxidation and oxidative products, and cell lysis.

To our knowledge, research on the antimicrobial properties of such molecules is usually conducted in aqueous media. With a view to developing coatings for building materials, we focused our evaluation methods to test the efficiency of MG as a support.

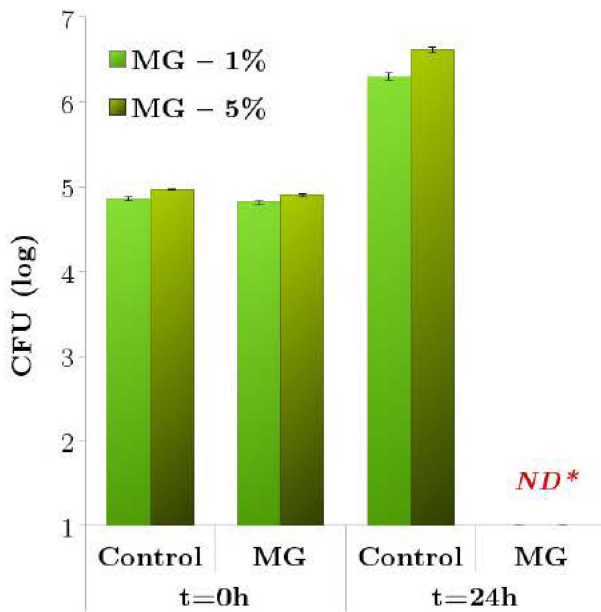


Fig. 2. Number of CFU (log₁₀) of *E. coli* CIP 53126 detected on samples during JIS Z 2801 assay. Mean \pm s.e., $n = 3$. ND*: Not detected.

3.2 Antibacterial activity of MG in coating form

The number of CFU (log₁₀) detected on samples just after the inoculation ($t = 0$ h) and after 24 h of test ($t = 24$ h) are presented in Figure 2. These results came from 2 experiments: one conducted with a 1% concentration of MG coating and one with a 5% concentration.

The numbers of CFU from control samples (noncoated membranes) and from MG-coated samples after a few seconds of contact ($t = 0$ h) were similar for both experiments. This indicates that the method used to separate bacteria from MG coating (filtration) is validated. Regarding results after 24 h of contact, control samples presented number of CFU 6.30 ± 0.04 logs and 6.61 ± 0.04 logs for the tests with 1% and 5% concentrations of MG, respectively. This indicates a bacterial growth of about 15 log ($p > 0.001$) compared to the values at $t = 0$ h.

No colonies were detected on samples that were coated with 1% or 5% of MG (the detection limit was 1 log). Corresponding antibacterial activities can be calculated for the two concentrations: $A_{1\%} \leq 5.62 \pm 0.04$ log and $A_{5\%} \leq 5.30 \pm 0.04$ log ($p < 0.001$ in both tests).

These results are consistent with the previous bactericidal test in liquid phase. The 1% concentration of MG was active on *E. coli* CIP 53126 after 24 h of contact, showing an inactivation of the inoculated microbial population as a whole. During inoculation step, the deposition of a drop of bacterial suspension on MG coating led to its dissolution in water. The antibacterial effect assessed in this experiment was the result of mechanisms that took place in an aqueous medium. These results are interesting but may not correspond to indoor environment conditions

in which construction materials are also targeted by airborne microorganisms.

3.3 Evaluation of MG coated on plasterboard

The last experiment of this study was carried out in order to observe the efficiency of MG as a coating to protect building materials from natural colonization, without any liquid microbial inoculation.

Recovery rates of microorganisms on plasterboard were obtained from image analysis (Fig. 3). Coated samples presented colonized areas of 2.6%, 10.6%, and 16.6% after 8, 10, and 14 weeks, respectively. Control samples (noncoated) presented colonized areas of 67.6%, 80.1%, and 91.1%.

Colonization patterns are presented in Figure 3. These images were used to calculate the percentages of colonized areas.

After 8 weeks, control samples (without MG) presented broad dark areas all over their surfaces SEM observations showed growth patterns that are characteristics features of fungal growth (Fig. 4). For comparison, uncontaminated plasterboards (coated with MG) are presented in Figure 5. MG-coated samples presented no contamination except for a few small spots near the edges of the samples (Fig. 3). These contamination spots seemed to spread slowly over the surface with time (weeks 8, 10 and 14). These results confirmed the antimicrobial properties of MG and its protective effect on building materials, even in severe conditions. The apparition of small amounts of contamination after 8 weeks on MG-coated samples may be explained by:

- a nonhomogeneous application of coating on the edges of samples,
- contamination from the bottom surface or from the edges of plasterboard samples, which were not completely coated,
- a loss of efficiency or a possible “consumption” of the MG molecules during the disinfection process: if the substance acts on cell membrane as has been suggested, it is possible that the molecule is either destroyed in the process or embedded in the bacterial membrane. This hypothesis emphasizes a non-residual effect of MG molecules on microorganisms.

Further work is required to support these hypotheses. Nevertheless, these results clearly attest to the antimicrobial properties of MG used as a coating for building materials. They also indicate that the antimicrobial properties are effective against airborne microbial contaminants as well as contaminants in aqueous media.

At present, mechanisms of action are not well known and their investigation will require specific tests. Previous researches have emphasized that fatty acids and fatty acid esters present antimicrobial activity targeting the cytoplasmic membrane of microorganisms [26, 29]. It seems likely that the activity is induced by surfactant properties of such molecules. The present study highlighted the capability of MG molecules to develop these properties when deposited on a substrate.

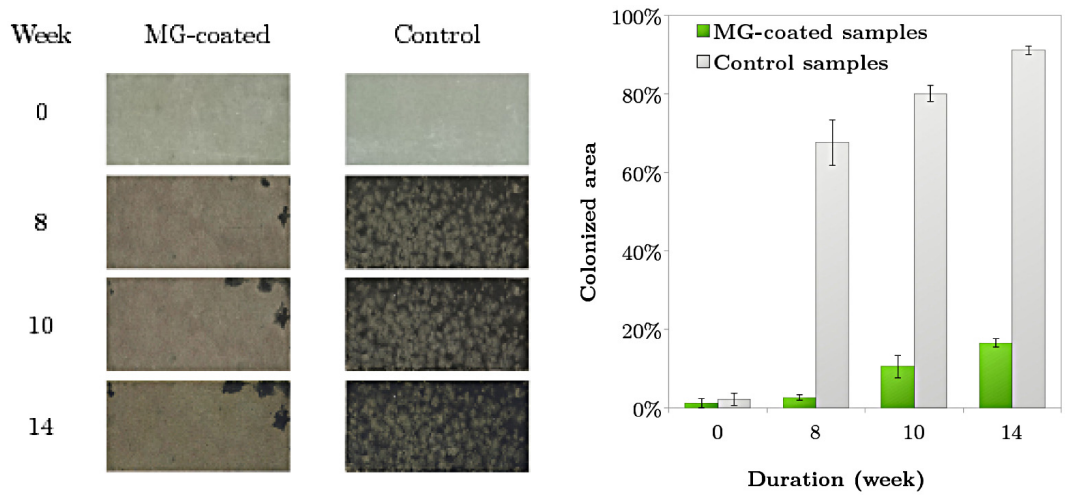


Fig. 3. LEFT: Plasterboard samples after 0, 8, 10, and 14 weeks of storage in a moisture chamber (100%RH, room temperature ≈ 20 °C); Right: Colonized area (%) of samples according to the incubation time. Mean \pm s.e., $n = 3$.

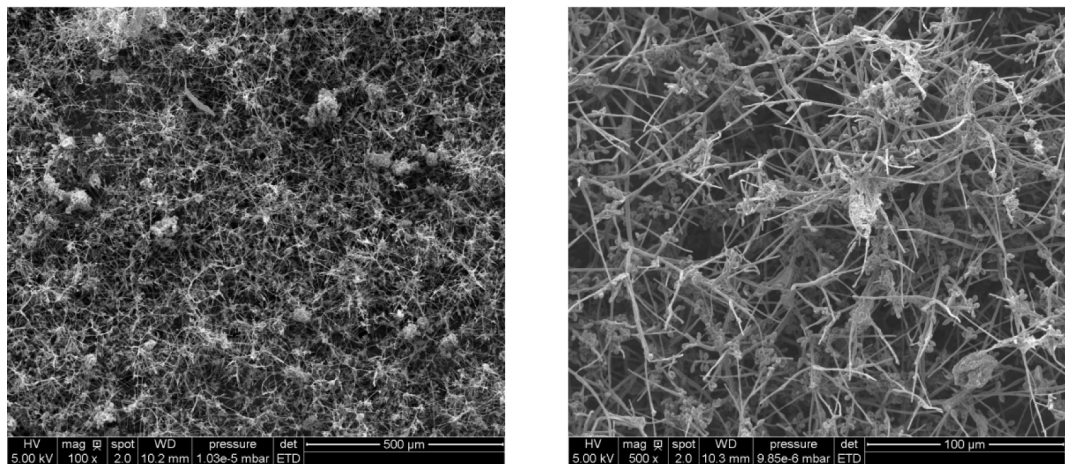


Fig. 4. SEM photographs (Jeol JSM-6380LV) of the microbial communities fouling plasterboard samples after 14 weeks. Left: magnification $\times 100$; Right: magnification $\times 500$.

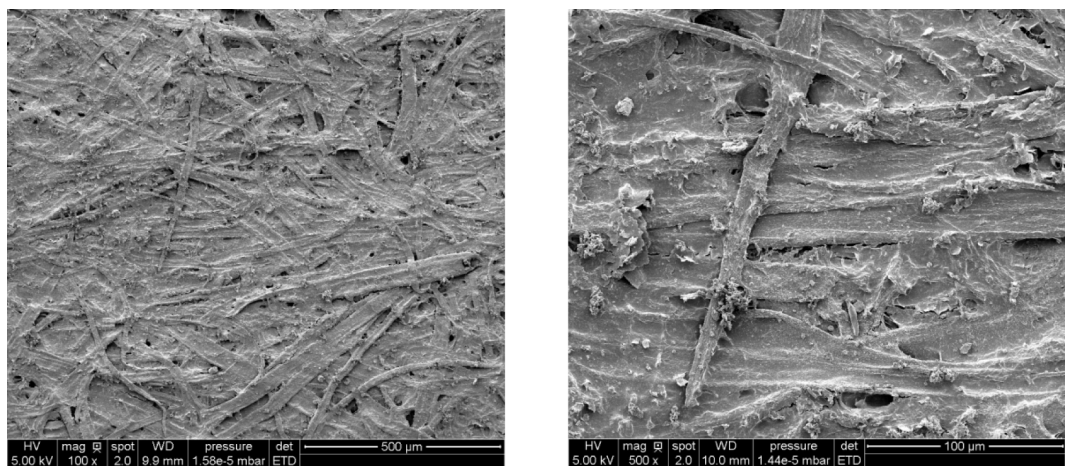


Fig. 5. SEM photographs (Jeol JSM-6380LV) of uncontaminated plasterboard samples after 14 weeks. Left: magnification $\times 100$; Right: magnification $\times 500$.

4 Conclusion and perspectives

This study focused on the antimicrobial properties of monoglyceride molecules. MG showed high bactericidal activities both in water and in the form of a coating for membranes. The application of a 1% concentration of MG in solution to a support is sufficient to inactivate about 5.6 log of *E. coli* in 24 h of contact. In addition, using MG as a coating limited natural microbial growth on building materials (plasterboard). These promising results in indoor conditions highlight the interest of using such environmentally friendly molecules resulting from the agro-industry. In addition, further research should explore:

- the antimicrobial spectrum (Gram negative/positive bacteria, fungi, etc.),
- the durability and behavior under environmental conditions. Regarding these aspects, stabilized MG molecules could be included in an acrylic coating. Studies should focus on improving (i) the retention of the coating on different types of support (mortar, plaster, wallpaper, etc.), and (ii) its resistance to abrasion and washing while maintaining its antimicrobial properties,
- the mechanisms of action of the molecule, including interactions with water molecules, potentially induced by water events on the material surface.

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