RESEARCH ARTICLE

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Antifungal Coating Based on Pyocyanin Nanoparticles (Np-Pyo)

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ABSTRACT

Pyocyanin is a pigment produced by 95% of *Pseudomonas aeruginosa* strains and exhibits antimicrobial properties that can be used for different purposes. In this work, PMMA-based nanoparticles that were encapsulated into 200 µg/mL of pyocyanin (Np-Pyo) were produced by the nanoprecipitation method. They were evaluated with respect to antifouling activity against *Aspergillus* sp. and *Penicillium* sp. With an encapsulation efficiency of 56%, the NpPyo remained stable for 90 days. Their characteristics were satisfactory for the following parameters: average size (616.90±38.30 nm; blank: 282.58±22.89 nm), polydispersion index (0.51±0.01; blank: 0.45±0.78), zeta potential (-5.13±0.41 mV; blank: -6.44±1.12 mV) and pH (6.18±0.03; blank: 6.42±0.01). The *in vitro* biofilm formation assay was performed on dolomite coupons measuring 1 cm², on which the formulation was applied. There were tested conditions with and without immersion for 72h at 30 ºC. In the tests with the immersed coupons, there was fungal colonization; this was, however, lower than that observed in the control. *A. niger* decreased by 3 log units. No growth was observed on the coupons that were not immersed. The results were promising and demonstrated viability as a means of antifouling protection, particularly on dry surfaces.

Keywords: Antifouling, bioreceptivity, coating, pyocyanin.

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I. Introduction

Bioreceptivity favors the formation of biofilms. It is a key to the process of biodeterioration of construction materials. This deterioration occurs in a dynamic, cumulative and non-uniform way related to the type of surface material colonized by microorganisms (Dias *et al*., 2021). The matrix that surrounds biofilms provides a resilient barrier of physical protection against hydrodynamic forces (Krsmanovic *et al.*, 2021), loss of water by evaporation (Cepas *et al*., 2019), as well as resistance to environmental stresses, such as lack of nutrients, salinity, pH changes and the presence of reactive and oxygen species (ROS) (Yin *et al.*, 2019).

Biodeterioration of construction surfaces is very costly in terms of prevention and repair of degraded material (Wei *et al*., 2013). Therefore, ways to prevent microbial colonization are recognized as valuable (Ünal, 2018). One of these strategies is the application of coatings (Ivanovna *et al.*, 2016). Paints and varnishes are coatings with a decorative or protective function that can be complemented with antimicrobial compounds such as algaecides (Gladis *et al.*, 2010), bactericides (Hoque *et al*., 2015) and/or fungicides (Heaton *et al*., 1991).

Biocides are compounds incorporated into coatings with the aim of delaying the natural process of deterioration in different types of materials (Schoknecht *et al*., 2009). Many substances are used as biocides, including antibiotics, metals, non-metals, cationic and non-cationic organic compounds (Cloutier et al., 2015). Many of these compounds, however, are synthetic, highly toxic, and not environmentally satisfactory (Carson *et al*., 2009), a limiting factor for concentrated use (Nowack & Bucheli, 2007). Furthermore, these biocides offer protection for a short time only (Nordstierna *et al*., 2010). Thus, the search for natural and biodegradable biocides is an attractive and effective prospect in the context of a sustainable world (Le Norcy *et al*., 2017). Additionally, the use of nanometric systems into which natural bioactives have been added have a longer life (Fiori *et al*., 2017), as the antifouling action works by

gradually releasing the active ingredient and reducing the leaching process (Jämsä *et al*., 2013).

Pyocyanin is a specific intense fluorescent blue pigment synthetized by about 95% of *Pseudomonas aeruginosa* strains (Gonçalves & Vasconcelos, 2021). The molecule exhibits properties against bacteria (Viana *et al*., 2017), filamentous fungi (Silva *et al*., 2020), yeasts (Özyürek *et al*., 2016) and protozoa (Marrez and Mohamed, 2020). The mechanism of action involves the formation and accumulation of ROS, especially superoxide and hydrogen peroxide. The result of this is the induction of oxidative stress and disruption of intracellular homeostasis, as well as reduction of the oxygen supply, interfering with the cellular respiration process (Jayaseelan *et al*., 2014).

The antimicrobial properties of pyocyanin demonstrate the bioprospecting potential of the molecule. Promising results have already been described and some attributes of pyocyanin have been applied, for example, as an agrochemical (de Britto *et al*., 2020; Khare & Arora, 2011), probiotic in aquaculture (Priyaja *et al*., 2014), antibiotic (Priyaja *et al*., 2016; Gharieb *et al*., 2013) and antitumor activities (Vipin *et al*., 2017). Thus, this present work proposed to apply pyocyanin, incorporated into nanoparticles to obtain a protective coat with antifouling property.

II. Experiment Methodology

A. Pyocyanin

Pyocyanin of 98% purity was purchased (Sigma-Aldrich, St. Louis, USA). The powder was recomposed in a 10% hydroethanolic solution and kept at -5°C. The same solution ($5x10^{-3}$ µg/mL pyocyanin) was used to design an analytical curve at 690 nm (IL 0082-Y-BI).

B. Nanoparticles

The pyocyanin nanoparticles (Np-Pyo) were prepared by the nanoprecipitation method (Fessi *et al*., 1989), with modifications. To compose the aqueous phase, 0.2 g of polyvinyl alcohol (PVA) (Sigma Aldrich, St Louis, USA) and distilled water were used. For the organic phase, 0.1 g of polymethylmethacrylate (PMMA) (Sigma Aldrich, St Louis, USA), acetone 99% as solvent, and 200 µg/mL of pyocyanin were used. After dissolution of the constituents, the organic phase was carefully dripped into the aqueous phase. The resulting suspension was evaporated under reduced pressure (Buchi R3), giving a final volume of 10 ml. Blank nanoparticles (without incorporation of pyocyanin) (Np-B) were prepared using the same protocol.

C. Determination of Pyocyanin Encapsulation Rate

The ultrafiltration-centrifugation method was used (Lima & Albuquerque, 2012). The total concentration of Np-Pyo (C1) was determined by dissolving the nanoparticles in distilled water. Briefly, an aliquot of 1 ml of the formulation was taken and inserted into a 10 ml volumetric flask. The mixture was homogenized using ultrasound (CD-3800, RM TecnoBrasil) for 15 minutes and then centrifuged at 4000xg for 40 minutes (CENTRIBIO 80-2B). The amount of pyocyanin in the supernatant was estimated by spectroscopy at 690 nm (Spectrophotometer IL 0082-Y-BI). The concentration of pyocyanin not associated with Np-Pyo (C2) was determined from the supernatant filtrate as a result of a centrifugation process (Chromafil – 0.45 µm) and subsequently estimated by spectrophotometry at 690 nm. The amount of encapsulated molecule was determined by calculating [(C1–C2) ÷ C1], multiplied by 100.

D. Characterization of Nanoparticles

Six characterization assays of Np-Pyo and Np-B were carried out. The average size and distribution of particles were determined by photon autocorrelation spectroscopy at 25ºC (Zetasizer nano ZS 90, Malvern) and are presented as the average of three measurements performed by the equipment. The images for observation of the morphology of the nanoparticles (Np-B and Np-Pyo) were produced by scanning electron microscopy (Zeiss Leo 1430). The zeta potential (ζ) was determined by electrophoretic mobility (Malvern ZS 90). The stability test verified the durability of the preparations for 90 days at regular time intervals. In the same intervals, the pH of the nanoparticle suspensions was determined (Tecnopon, mPA 210).

E. Filamentous Fungi

Two wild specimens, *Aspergillus niger* and *Penicillium* sp., recovered from an outer wall, were used (Silva *et al*., 2020). The fungal susceptibility to pyocyanin was observed by Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC). Briefly, the assays were carried out in a microdilution plate containing 100 µL of doubly concentrated Sabouraud-Dextrose broth 2% (SDB 2%), 100 µL of the pyocyanin solution (concentrations ranged among 140 and 8.75 µg/mL) and 10 µL of spore suspension (10^6 CFU/mL), prepared in 0.9% NaCl solution. The microplates were incubated at 30ºC for 48 h. The MIC was described as the lowest dilution at which no fungal growth was found by visual inspection. The MFC was identified by transferring 10 µL from the MICx2, MIC and MIC÷2 wells to new microplates containing SDB 2%. After incubation at 30ºC for 48 h, MFC was described as the pyocyanin value where there was no turbidity, observed by visual inspection. The test controls verified the viability of the fungi in the SDB 2%, as well as the sterility of the broth. The assays were performed in triplicate (Silva *et al*., 2018).

F. Inocula Preparation

Spore suspensions of *Aspergillus niger* and *Penicillium* sp. were prepared from fresh cultures incubated on Sabouraud-Dextrose agar 2% at 30°C for 7-14 days. They were prepared in 5 ml of sterile 0.9% NaCl solution, added to 50 µL of Tween 80 (Neon, São Paulo, Brazil). Then, the suspensions were shaken for 2 minutes (Warmnest, W28), and 50 µL transferred to a Neubauer chamber (Labor) and approximately 1-3x106 spores/mL were obtained (CLSI, 2002).

G. In vitro Biofilm Formation Assay

Dolomite coupons (Legato, Rio de Janeiro, Brazil) were used; these had a rough surface with a minimum area of 1 cm^2. About 2 mL of the Np-Pyo and Np-B suspension were aseptically applied to the center of the coupons and carefully spread with a brush. The coupons were kept in a sterile environment for 48 hours. Afterwards, two conditions

were assessed. In the first one, the short-term immersion test was used. The coupons were aseptically immersed in 18 ml of SDB 2% and 2 mL of the spore suspension prepared as described above. Under the second condition, the coupons were inoculated with 2 mL of the spore suspension in SDB 2% diluted 10 times. Then the coupons were maintained in Petri dishes (Van Dijck *et al*., 2018) and incubated at 30ºC for 72h. In the control test, the dolomite coupon was used without coating, inoculated with the spore suspension in SDB 2% diluted 10 times.

The quantification of cells adhered to the surface of the coupons was carried out by determining the Most-Probable-Number (MPN) (Dias *et al*., 2016). Briefly, the surface nanoparticle coating was carefully scraped into a 10 ml sterile 0.9% NaCl solution. After homogenization, the suspension was diluted to 10^{-3}; then 100 µL from each tube was transferred to microplates containing the same volume of doubly concentrated SDB 2%. The microplates were incubated for 72 h at 30ºC. The MPN per 100 µL/cm^2 was determined by counting the wells in which there was growth, indicated by the turbidity, and then comparing them to the MPN Table. The test was performed in triplicate.

III. Results

A. Characterization of the Formulation Containing Pyocyanin

The Np-Pyo presented a macroscopically homogeneous appearance. Encapsulation efficiency of the nanosystems was 56%. Under static conditions, the formulation containing Np-Pyo was composed of two phases that rapidly homogenized (Fig. 1).

Over 90 days, no contamination was observed, and the systems containing Np-Pyo and Np-B remained stable. Means of Np-Pyo size of 616.9±38.3 nm (Np-B: 282.6±22.9 nm), polydispersity index (PDI) of 0.51±0.01 (Np-B: 0.451±0.001) were observed. 0.176), zeta potential (ζ) of -5.13±0.41 mV (Np-B: -6.44±1.12 mV) and pH of 6.18±0.03 (Np-B: 6, 42±0.01) (Table I).

The SEM images enabled a better view of the distribution of the nanoparticles, as well as their homogeneity (Fig. 2).

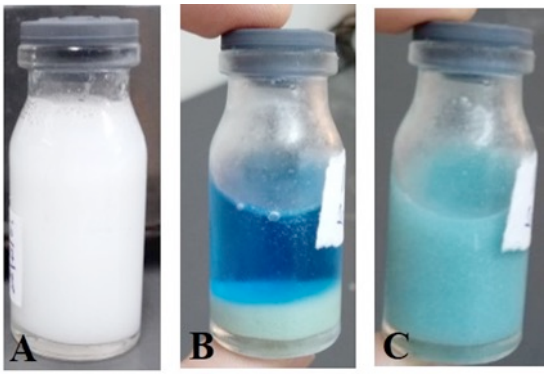

Fig. 1. Macroscopic aspects of the formulation of Np-B (A) and Np-Pyo: static state (B) and homogenized (C)

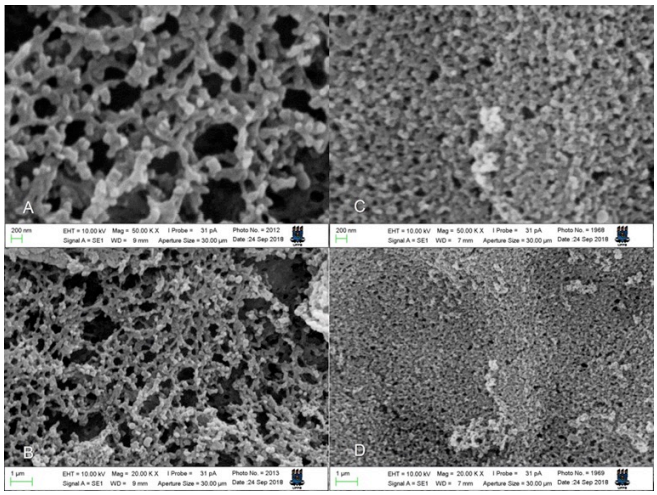

Fig. 2. Scanning electron microscope image of Np-Pyo (A e B) and Np-B (C e D) represented in nano and micro scale (C)

TABLE I: CHARACTERISTICS OF NANOSYSTEMS OVER 90 DAYS

Np-Pyo					
Time (day)	pH (±0.01)	Size (±0.30 nm)	PDI (±0.10)	Z (±0.10 mV)	Macroscopic characteristics
0	6.14	668.70	0.34	-5.35	Blue, milky, with precipitates
7	6.18	---	---	---	
15	6.14	621.80	0.53	-4.40	
21	6.18	---	---	---	
30	6.18	581.90	0.58	-5.18	Blue, with two phases that homogenized under agitation
60	6.22	595.30	0.58	-5.33	
90	6.21	593.80	0.51	-5.37	

Np-B					
Time (day)	pH (±0.01)	Size (±0.10 nm)	PDI (±0.10)	Z (±0.10 mV)	Macroscopic characteristics
0	6.42	273.83	0.28	-7.76	White, milky, homogeneous
7	6.42	---	---	---	
15	6.42	291.36	0.42	-5.72	
21	6.42	---	---	---	
30	6.45	286.76	0.43	-7.53	White, with two phases that homogenized under agitation
60	6.41	249.53	0.38	-5.85	
90	6.39	311.43	0.75	-5.33	

B. Antifungal activity of Np-Pyo

Initially, the activity of pyocyanin against fungi was verified. Under the test conditions, MIC was 140 µg/mL for both *Aspergillus niger* and *Penicillium* sp. MFC was estimated to be > 140 µg/mL, the upper limit of the test. Because of this, we then encapsulated 30% more pyocyanin MIC.

In the short-term immersion test, fungal colonization was observed on the dolomite surface, but the number of cells was reduced when compared to the control. *Penicillium* sp. was estimated at 160 MPN/100µL/cm^2 (control, 23x10^3 MPN/100µL/cm^2) while *A. niger* was 360 MPN/100µL/cm^2 (control, >23x10^4 MPN/100µL/cm^2), indicating a biocidal effect of pyocyanin on *A. niger*.

In the test with the non-immersed coupons, the Np-Pyo was effective, inhibiting the fungal growth. For the coupons that received the coverage, there was fungi growth only along the edges, with *Penicillium* sp. apparently being the most sensitive fungus, based on observation of more aerial mycelia on the coupons inoculated with *A. niger*. The control coupons without coverage suffered growth from both fungi over their entire surface (Fig. 3).

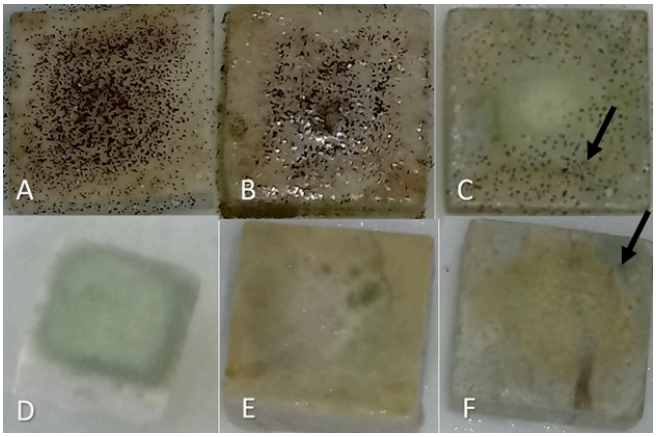

Fig 3. Developmental aspects of *Aspergillus niger* (A-C) and *Penicillium* sp. (D-F) in the dolomite coupons of the test without immersion: control (left), blank (center) and Np-Pyo (right). Aerial mycelia were observed along the edges, as indicated by arrows.

IV. Discussion

A. Pyocyanin nanoparticles

Pyocyanin is not the first naturally occurring active material to be incorporated into controlled-release systems in roof coverings to protect the constructions beneath (Palanichamy & Subramanian, 2017; Trojer *et al*., 2015). However, as far as we know this is the first report of the formulation of Np-Pyo being incorporated into a material used as an antifungal coating. Nanoparticles have been successfully obtained by nanoprecipitation. This is considered an important method in the production of nanosystems for conveying active substances where hydrophilic and hydrophobic actives are combined (Rao & Geckeler, 2011). In addition, the non-formation of aggregates was due to the choice of stabilizer. This was important because PMMA is hydrophobic. With the use of PVA, solubilization of the polymer in water was possible (Nordstierna *et al*., 2011; Nordstierna *et al*., 2010).

The encapssulation rate of Np-Pyo was 56%, considered low. Drug entrapment efficiency can occur at rates that range between 65 and 90% (Lekshmi *et al*., 2010), however, the percentage achieved in the present study did not prejudice the entrapment. Further adjustments may improve the binding capacity between pyocyanin and the polymer. Pyocyanin is a water-soluble pigment. This characteristic may have contributed to the loss to the aqueous phase during the production process (Niwa *et al*., 1993).

The characterization parameters of Np-Pyo showed positive findings. SEM images revealed a satisfactory shape of the particles when placed on the surface. In addition, Np-Pyo was stable over the long term, desirable in nanosystems. The evaluation of stability is essential because it considers factors such as potential alteration arising from external additives added to the formulation, as well as the chemical interaction between the active compounds and the components of the formulation (Schaffazick *et al*., 2003).

A subtle increase in pH was observed in the formulations containing Np-Pyo, compared to Np-B, which was more stable. The change in pH may be an indication of polymer degradation (Guterres *et al*., 1995), however, the increase in pH of the formulation containing Np-Pyo could have been more related to the alkaline property of pyocyanin (Ohfuji *et al*., 2004). Regarding the zeta potential (ζ), the results were within the desired stability range, that is, values above +30 mV or below -30 mV. Zeta Potential is a useful indicator of net particle surface charge and can be used to predict and control the stability of colloidal suspensions and emulsions (Wongsagonsup *et al*., 2005).

Particle size is often used to characterize nanosystems because it facilitates the understanding of subsequent dispersion and aggregation (Birnbaum *et al*., 2000). The literature reports that a great variation can be observed between nano and micrometric particle sizes when PMMA is used as a polymer (Siddiqui *et al*., 2018). Additionally, the differences between the sizes of Np-Pyo and Np-B may be related to their incorporation into the organic phase with PMMA, which may decrease the viscosity of this phase, facilitating the diffusion to the aqueous phase. This leads to a decrease in droplet formation, culminating in a reduction in the average particle size (Aubry *et al*., 2009).

The PDI values presented variation over time. The PDI identifies the homogeneity in the population of particles (Liu & Chen, 2009). Values above 0.3 however, suggest a polymodal distribution of the species, i.e., when there is more than one type of particle population (Gaumet *et al*., 2008). This variation of the Np-Pyo PDI indicated an increase in the aggregation of the particles due to the hydrophobic nature of PMMA. However, it is important to note that the polymodal distribution for an ink or a coating would not affect the result, unlike what is required for a drug (Apostol *et al*., 2005).

Further studies may improve aspects related to optimization of the encapsulation efficiency, in order to contain a greater amount of pyocyanin associated with the polymeric wall. In addition, new research can be evaluated in tests aimed, for example, at the use of the coating containing Np-Pyo as a base applied before painting or even used as an additive in paint compositions to replace the toxic and less biodegradable compounds used today.

B. Antifungal activity of Np-Pyo

The understanding of the effectiveness of biocides incorporated into coatings on the formation of fungal biofilms is an issue that still needs to be evaluated (Stirling *et al*., 2011). Filamentous fungi can colonize on indoor walls (Richardson & Rautemaa-Richardson, 2019) as well as on those outdoors (Ogawa *et al*., 2017). The most prevalent organisms found are ascomycete species, especially of the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma* and *Trichophyton* (Shirakawa *et al*., 2010; Gaylarde & Gaylarde, 2005; Adeleye & Adeleye, 2000). These fungi are recognized as anemophiles and can form biofilms as a survival strategy under unfavorable conditions, such as those found on building surfaces (Harding, 2009). Additionally, *Aspergillus* and *Penicillium* are two among the most representative genera of opportunistic pathogens related to indoor diseases (Sánchez-Espinosa *et al*., 2021; Pitt, 1994).

Fungal colonization in constructions is a topic of interest because it deals with bioreceptivity phenomena, responsible for the deterioration of these materials, causing different kinds of pathologies (Manso *et al*., 2014; Miller *et al*., 2012). Surface biodeterioration occurs mainly due to the production of organic acids and the penetration of hyphae (de la Torre *et al*., 1993).

Aesthetically, the colonization of fungi can cause damage to historical buildings (Palko & Deáková, 2014). Depending on the construction, this poses a threat to the conservation of valuable historical and cultural heritage edifices (Cappitelli *et al*., 2020; Gallego-Cartagena *et al*., 2020).

Epidemiologically, buildings and monuments colonized by fungi also represent public health problems (Wang *et al*., 2018), particularly respiratory diseases, especially aspergillosis (Mousavi *et al*., 2016). Moreover, other important conditions are related to the permanence of humans and other vertebrates in dwellings contaminated by opportunistic fungi, such as allergies (Żukiewicz-Sobczak, 2013), dermatophytosis (Singh *et al*., 2009), sick building syndrome (Sun *et al*., 2019) and mycotoxicoses (Aleksic *et al.*, 2017). Even so, many diseases or conditions caused by exposure to fungi are underreported (Zock *et al*., 2002), unidentified or even confused with other pathologies (Burge, 2002).

Pyocyanin is a secondary metabolite involved in natural ecological phenomena of antibiosis and amensalism of *P. aeruginosa* against its competitors (Jameel *et al*., 2017; Ghoul & Mitri, 2016). Depending on the concentration of pyocyanin, as well as the susceptibility of the organism exposed to the substance, a biocidal or biostatic effect may result (Arruda *et al*., 2020). Due to the antimicrobial properties and wide spectrum of action of pyocyanin (Vasconcelos *et al*., 2010), the molecule is a promising naturally active compound with potential and physicochemical characteristics that may be incorporated into nanosystems for the formulation of coatings.

Pyocyanin is a planar molecule with low molecular weight and has hydrophilic and hydrophobic properties (Baron & Rowe, 1981). The use of nanoscale technology is interesting when greater solubility of active substances is desired, achieving the effect when using a lower concentration, combined with a prolonged release of the active and, consequently, providing protection against oxidation and physical-chemical degradation (Paiva-Santos *et al*., 2021).

In the test without immersion of dolomite coupons, Np-Pyo prevented the growth of fungi, indicative of a biocidal effect on both *A. niger* and *Penicillium* sp. Additionally, the fungicidal effect was observed by the reduction of 3 log units of the MPN of *A. niger* compared to the control in the short-term immersion test, demonstrating that this fungus was more sensitive to Np-Pyo than *Penicillium* sp, indicating that the encapsulation had potentiated the effects of pyocyanin.

Previous studies of free pyocyanin activity observed a biostatic effect against filamentous fungi, such as *Aspergillus flavus*, *A. fumigatus* (Sudhakar *et al*., 2013), *Fusarium* sp. (Afzal *et al*., 2013) and *Tricophyton rubrum* (El-Zawany & Ali, 2016). A similar fungistatic effect has also been reported in yeasts, *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* (Bonifácio *et al*., 2020) and *Cryptococcus neoformans* (Karpagam *et al*., 2013).

According to the results of this study, it is suggested that the biofouling observed with *Penicillium* sp. was mediated by a fungistatic action of pyocyanin. It may be driven by a possible physicochemical instability of Np-Pyo caused by the conditions used in the assay (Lyra *et al*., 2021). When PMMA is subjected to aqueous systems, its hydrophobicity may promote turgor pressure of the nanosystem causing the biocide to be leached, resulting in the reduction or cessation of its activity (Furno *et al*., 2004). This may explain the fact that even *Penicillium* sp. reduced the MPN/µL/cm^2. Compared to the control, this reduction was less than 3 log units, indicating a biostatic effect.

V. Conclusion

The study presented as far as we know is the first formulation of Np-Pyo for application as a coating whose objective is to reduce fungal colonization. The nanosystems showed satisfactory long-term stability, indicating a promising application of pyocyanin as antifungal coating on dry surfaces. Further studies must be carried out in order to provide means to better use entrapped pyocyanin.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

REFERENCES

Adeleye I. A., & Adeleye O. A. (2000). Isolation and identification of microbes associated with paints and weathered painted walls. *Journal of Scientific Research and Development*, 4, 71-76.

Aleksic B., Draghi M., Ritoux S., Bailly S., Lacroix M., Oswald I. P., et al. (2017). Aerosolization of mycotoxins after growth of toxinogenic fungi on wallpaper. *Applied and Environmental Microbiology*, 83(16): e01001-17, doi: 10.1128/AEM.01001-17.

Apostol M., Baret P., Serratrice G., Desbrières J., Putaux J-L., Stébé M-J., et al. (2005). Self-assembly of an amphiphilic iron(iii) chelator: mimicking iron acquisition in marine bacteria. *Angewandte Chemie,* 117(17): 2636-2638.

Arruda R. R. A., Oliveira B. T. M., Bonifácio T. T. C., Morais V. C., Amaral I. P. G., & Vasconcelos U. (2020). Activity of two exometabolites produced by *Escherichia coli* on the synthesis of pyocyanin. *International Journal of Advanced Engineering Research Science*, 6: 267–271.

Aubry J., Ganachaud F., Addad J-P. C., & Cabane B. (2009). Nanoprecipitation of polymethylmethacrylate by solvent shifting: 1. boundaries. *Langmuir*, 25(4): 1970-1979.

Baron S. S., & Rowe J. J. (1981). Antibiotic action of pyocyanin. *Antimicrobial Agents and Chemotherapy*, 20: 814–820.

Bonifácio T. T. C., Arruda R. R. A., Oliveira B. T. M., Silva J. E. G., & Vasconcelos U. (2020). Exposure to pyocyanin promotes cellular changes in *Candida* spp. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 11: 111–119.

Burge A. (2002). An update on pollen and fungal spore aerobiology. *Journal of Allergy and Clinical Immunology*, 110(4): 544-552.

Birnbaum D. T., Kosmala J. D., & Branno-Peppas L. (2000). Optimization of preparation tecniques for poly (lactic acid-co-glycolic acid) nanoparticles. *Journal of Nanoparticle Research*, 2: 173–181.

Cappitelli F., Cattò C., & Villa F. (2020). The control of cultural heritage microbial deterioration. *Microorganisms*, 8(10):1542, doi: 10.3390/microorganisms8101542.

Carson R. T., Damon M., Johnson L. T., & Gonzalez J.A. (2009). Conceptual issues in designing a policy to phase out metal-based antifouling paints on recreational boats in San Diego Bay. *Journal of Environmental Management*, 90: 2460–2468.

Cepas V., López Y., Muñoz E., Rolo D., Ardanuy C., Martí S., et al. (2019). Relationship between biofilm formation and antimicrobial resistance in Gram-negative bacteria. *Microbial Drug Resistance*, 25(1): 72-79.

Cloutier M., Mantovani D., & Rosei F. (2015). Antibacterial coatings: Challenges, perspectives, and opportunities. *Trends in Biotechnology*, 33(11): 637-652.

CLSI. (2002). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. Wayne: CLSI.

De Britto S., Gajbar T. D., Satapute P., Sundaram L., Lakshmikantha R. Y., Jogaiah S., et al. (2020). Isolation and characterization of nutrient dependent pyocyanin from *Pseudomonas aeruginosa* and its dye and agrochemical properties. *Scientific Reports*, 10: 1542.

De la Torre M. A., Gomez-Alarcon G., Vizcaino C., & Garcia M.T. (1993). Biochemical mechanisms of stone alteration carried out by filamentous fungi living in monuments. *Biogeochemistry*, 19: 129-147.

Dias S. B. D., Jaramillo L. Y. A., Guedes D., Duran R., Carbon A., Bertolino L. C., et al. (2021). Assessment of acid mist on mortar biodeterioration simulating the wall of Jardim da Princesa, the National Museum of Rio de Janeiro, Brazil. *International Biodegradation and Biodeterioration*, 157: 105155, doi: 10.1016/j.ibiod.2020.105155.

Dias D. S. B., Vasconcelos U., Lutterbach M. T. S., Cravo-Laureau C., & Sérvulo E. F. C. (2016). Sessile aerobic microbiota from the wall of the National Museum, Brazil: characterization and quantification. *Canadian Journal of Pure and Applied Science*, 10(3): 3941-3949.

El-Zawawy N. A., & Ali S. S. (2016). Pyocyanin as anti-tyrosinase and anti tinea corporis: A novel treatment study. *Microbial Pathogenesis*, 100: 213-220.

Fessi H., Puisieux F., Devissaguet, J-Ph., Ammoury N., & Benita S. (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics*, 55(1): R1-R4.

Fiori J. J., Silva L. L., Picolli K. C., Ternus R., Ilha J., Decalton F., et al. (2017). Zinc oxide nanoparticles as antimicrobial additive for acrylic paint. *Material Science Forum*, 899: 248-253.

Furno F., Morley K. S., Wong B., Sharp B. L., Arnold P. L., Howdle S. M., et al. (2004). Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? *Journal of Antimicrobial Chemotherapy*, 54:1019-1024.

Gallego-Cartagena E., Morillas H., Maguregui M., Patiño-Camelo K., Marcaida I., Morgado-Gamero W., et al. (2020). A comprehensive study of biofilms growing on the built heritage of a Caribbean industrial city in correlation with construction materials. *International Biodegradation and Biodeterioration*, 147: 104874, doi: 10.1016/j.ibiod.2019.104874.

Gaumet M., Vargas A., Gurny R., & Delie F. (2008). Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. *European Journal of Pharmaceutics and Biopharmaceutics*, 69: 1-9.

Gaylarde C. G., & Gaylarde P. M. (2005). A comparative study of the major microbial biomass on exterior buildings in Europe and Latin America. *International Biodeterioration and Biodegradation*, 55(2): 131-139.

Gharieb M. M., El-Sheekh M. M., El-Sabbagh S., & Hamza W.T. (2013). Efficacy of pyocyanin produced by *Pseudomonas aeruginosa* as a topical treatment of infected skin of rabbits. *Biotechnology: An Indian Journal*, 7: 184–193.

Ghoul M., & Mitri S. (2016). The ecology and evolution of microbial competition. *Trends in Microbiology*, 24(10,): 833-845.

Gladis F., Eggert A., Karsten U., & Schumann R. (2010). Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles. *Biofouling*, 26(1): 89-101.

Gonçalves T., & Vasconcelos U. (2021). Colour me blue: The history and biotechnological potential of pyocyanin. *Molecules*, 26: 927, doi: 10.3390/molecules26040927.

Guterres S. S., Fessi H., Barra G., Devissaguet J-Ph., & Puisieux F. (1995). Poly(D-L-lactide) nanocapsule containg diclofenac: I. formulation and stability study. *International Journal of Pharmaceutics*, 113: 57-63.

Harding M. W., Marques L. L. R., Howard R. J., & Olson M. E. (2009). Can filamentous fungi form biofilms? *Trends in Microbiology*, 17(11): 475-480.

Heaton P. E., Callow M. E., Butler G. M., & Milne A. (1991). Control of mould growth by anti-fungal paints. *International Biodeterioration*, 27(2): 163-173.

Hoque J., Akkapeddi P., Yadav V., Manjunath G. B., Uppu D. S. S. M., Konai M. M., et al. (2015). Broad spectrum antibacterial and antifungal polymeric paint materials: synthesis, structure–activity relationship, and membrane-active mode of action. *ACS Applied Materials & Interfaces*, 7(3): 1804-1815.

Ivanovna L. V., Viktorovna M. L., & Viktorovich T. R. (2016). Method of assessment quality protective and decorative coating concrete cement. *Case Studies in Construction Materials*, 4: 81-84.

Jämsä S., Mahlberg R., Holopainen U., Ropponen J., Savolainen J., & Ritschkoff A. C. (2013). Slow release of a biocidal agent from polymeric microcapsules for preventing biodeterioration. *Progress in Organic Coatings*, 76: 269– 276.

Jameel Z. J., Hussain A. F., Al-Mahdawi M. A., Alkerim N. F. A., & Alrahman E. S. A. (2017). Bioactivity of pyocyanin of *Pseudomonas aeruginosa* clinical isolates against a variety of human pathogenic bacteria and fungi species. *The International Arabic Journal of Antimicrobial Agents*, 7: 2, doi: 10.3823/812

Jayaseelan S., Ramaswamy D., & Dharmaraj S. (2014). Pyocyanin: Production, applications, challenges and new insights. *World Journal of Mirobiology and Biotechnology*, 30: 1159–1168.

Sánchez-Espinosa, K. C., Rojas-Flores T. I., Davydenko, S. R., Venéro-Fernández S. J., & Almaguer, M. (2021). Fungal populations in the bedroom dust of children in Havana, Cuba, and its relationship with environmental conditions. *Environmental Science Pollution Research*, 28: 53010–53020, doi: 10.1007/s11356-021-14231-8.

Khare E., & Arora N. K. (2011). Dual activity of pyocyanin from Pseudomonas aeruginosa – Antibiotic against phytopathogen and signal molecule for biofilm development by rhizobia. *Canadian Journal of Microbiology*, 57: 708–713.

Krsmanovic M., Biswas D., Ali H., Kumar A., Ghosh R., & Dickerson A. K. (2021). Hydrodynamics and surface properties influence biofilm proliferation. *Advances in Colloid and Interface Science*, 288: 102336, doi: 10.1016/j.cis.2020.102336.

Le Norcy T., Niemann H., Proksch P., Linossier I., Vallé-Réhel K., Hellio C., et al. (2017). Anti-Biofilm effect of biodegradable coatings based on hemibastadin derivative in marine environment. *International Journal of Molecular Science*, 18(7): 1520, doi: 10.3390/ijms18071520.

Lekshmi U. M. D., Poovi G., Kishore N., & Reddy P. N. (2010). *In vitro* characterization and in vivo toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. *International Journal of Pharmaceutics*, 396(1-2): 194-203.

Lima M. L., & Albuquerque C. N. (2012). *Preparo e caracterização de nanopartículas de poli(ε-caprolactona) contendo megazol, agente anti-chagásico*. Rev Bras Farm 93(4): 493-498. Portuguese.

Liu H., & Chen J. (2009) Indomethacin-loaded poly (butylcyanoacrylate) nanoparticles: preparation and characterization. *PDA Journal of Pharmaceutical Science and Techonology*, 63: 207-216.

Lyra A. M., Ribeiro J. P. M., Nadal J. M., Baglie S., Klein T., Novatski A., et al. (2021). Efavirenz-loaded polymeric nanocapsules: Formulation, development, and validation of an RP-UHPLC-DAD method for drug quantification, determination of encapsulation efficiency, stability study, and dissolution profile. *Journal of Applied Pharmaceutical Science*, 11(2): 93-101.

Manso S., de Muynck W., Segura I., Aguado A., Steppe K., Boon N., et al. (2014). Bioreceptivity evaluation of cementitious materials designed to stimulate biological growth. *Science of the Total Environment*, 481: 232–241.

Marrez D. A., & Mohamad H. S. (2020). Biological activity and applications of pyocyanin produced by *Pseudomonas aeruginosa*. *Open Access Journal of Biomedical Science*, 1(4): 140-144.

Miller A. Z., Sanmartín P., Pereira-Pardo L., Dionísio A., Saiz-Jimenez C., & Macedo M. F. (2012). Bioreceptivity of building stones: a review. *Science of the Total Environment*, 426: 1–12.

Mousavi B., Hedayati M. T., Hedayati N., Ilkit M., & Syedmousavi S. (2016). *Aspergillus* species in indoor environments and their possible occupational and public health hazards. *Current Medical Mycology*, 2(1): 36-42.

Niwa T., Takeuchi H., Hino N., & Kunou Y. (1993). Preparation of biodegradable nanospheres of water soluble and insoluble drugs with dl-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *Journal of Control Release*, 25: 89–98.

Nordstierna L., Abdalla A. A., Masuda M., Skarnemark G., & Nydén M. (2010). Molecular release from painted surfaces: Free and encapsulated biocides. *Progress in Organic Coatings*, 69: 45-48.

Nordstierna L., Movahedi A., & Nydén, M. (2011). New route for microcapsule synthesis. *Journal of Dispersion Science and Technology*, 32(3): 310-311.

Nowack B., & Bucheli, T.D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution*, 150(1): 5-22.

Ogawa A., Celikkol-Aydin S., Gaylarde C., Baptista-Neto J. A., & Beech I. (2017). Microbial communities on painted wet and dry external surfaces of a historic fortress in Niterói, Brazil. *International Biodegradation and Biodeterioration*, 123: 164-173.

Ohfuji K., Sato N., Hamada-Sato N., Kobayashi T., Imada C., Okuma H., et al. (2004). Construction of a glucose sensor based on a screen-printed electrode and a novel mediator pyocyanin from *Pseudomonas aeruginosa*. *Biosensors and Bioelectronics*, 19: 1237-1244.

Özyürek S. B., Gür S. D., & Bilkay I. S. (2016). Investigation of antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* strains isolated from different clinical aspecimens. *Hacettep Journal of Biology and Chemistry*, 44: 1–6.

Paiva-Santos A. C., Mascarenhas-Melo F., Coimbra S. C., Pawar K. D., Peixoto D., Chá-Chá R., et al. (2021). Nanotechnology-based formulations toward the improved topical delivery of anti-acne active ingredients. *Expert Opinion on Drug Delivery*, 18(10): 1435-1454.

Palanichamy S., & Subramanian G. (2017). Antifouling properties of marine bacteriocin incorporated epoxybased paint. *Progress in Organic Coatings*, 103: 33-39.

Palko M., Deáková K. (2014). Aesthetic and technical problems of renovated residential buildings facade - prevention of defects. *Advances Material Research*, 855: 62-66.

Pitt J. I. (1994). The current role of *Aspergillus* and *Penicillium* in human and animal health. *Journal of Medical and Veterinary Micology*, 32(1): 17-32.

Priyaja P., Jayesh P., Haseeb M., Jose B., Phillip R., & Bright Singh I. S. (2013). Evaluation of pyocyanin toxicity in various life stages of *Penaeus monodon* and in nitrifying bacterial consortia for their safe application in recirculating aquaculture systems (RAS) to abrogate pathogenic vibrios. *Aquaculture International*, 25: 743–753.

Priyaja P., Jayesh P., Philip R., & Singh I. S. B. (2016). Pyocyanin induced in vitro oxidative damage and its toxicity level in human, fish and insect cell lines for its selective biological application. *Cytotechnology*, 68: 143–155.

Rao J. P., & Geckeler K. E. (2011). Polymer nanoparticles: Preparation techniques and size-control parameters. *Progress in Polymer Science*, 36: 887-913.

Richardson M., & Rautemaa-Richardson R. (2019). Exposure to *Aspergillus* in home and healthcare facilities' water environments: focus on biofilms. *Microorganisms*, 7(1): 7, doi:10.3390/microorganisms7010007.

Schaffazick R. S., Guterres S. S., Freitas L. L., & Pohlmann R. A. (2003). *Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos*. Quim Nova 26: 726-737. Portuguese.

Schoknecht U., Gruycheva J., Mathies H., Bergmann H., & Burkhardt M. (2009). Leaching of biocides used in façade coatings under laboratory test conditions. *Environmental Science and Technology*, 43(24): 9321-9328.

Shirakawa M. A., Tavares R. G., Gaylarde C. C., Taqueda M. E. S., Loh K., & John V.M. (2010). Climate as the most important factor determining anti-fungal biocide performance in paint films. *Science of the Total Environment*, 408(23): 5878-5886.

Siddiqui N., Bhardwaj A., Hada E., Yadav V. S., & Goyal D. (2018). Synthesis, characterization and antimicrobial study of poly (methyl methacrylate)/Ag nanocomposites. *Vacuum*, 153: 6-11.

Silva J. E. G., Bonifácio T. T. C., Arrruda R. R. A., Kretzschmar E. A. M., & Vasconcelos U. (2020). *Atividade bioestática da piocianina sobre fungos filamentosos isolados de parede de concreto*. Int J Develop Res 10: 35230–35264. Portuguese.

Silva D. F., Silva A. C. L., Diniz Neto H., Oliveira H. M. B. F., Medeiros C. I. S., Pereira J. A., et al. (2018). Activity anti-Candida albicans and effects of the association of β-citronellol with three antifungal azolics. *Latin American Journal of Pharmacy*, 1: 182-190.

Singh I., Mishra A., & Kushwaha R. K. S. (2009). Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals. *Indian Journal of Medical Microbiology*, 27(3): 242-246.

Stirlin R., Uzunovic A., & Morris P.I. (2011). Control of black stain fungi with biocides in semitransparent wood coatings. *Forest Products Journal*, 61(5): 359-364.

Sun Y., Hou J., Cheng R., Sheng Y., Zhang X., & Sundell J. (2019). Indoor air quality, ventilation and their associations with sick building syndrome in Chinese homes. *Energy and Buildings*, 197: 112-119.

Trojer M. A., Nordstierna L., Bergek J., Blanck H., Holmberg K., & Nydén M. (2015). Use of microcapsules as controlled release devices for coatings. *Advances in Colloid Interface Science*, 222: 18-43.

Ünal H. (2018). Antibiofilm coatings. In Tiwari A. (Ed.) *Handbook of antimicrobial Coatings*. (pp. 301-319). Elsevier.

Van Dijck P., Sjollema J., Cammue B. P. A., Lagrou K., Berman J., d'Enfert C., et al. (2018). Methodologies for *in vitro* and *in vivo* evaluation of efficacy of antifungal and antibiofilm agents and surface coatings against fungal biofilms. *Microbial Cell*, 5(7): 300-326.

Vasconcelos U., Lima M. A. G. A., & Calazans G. M. T. (2010). *Pseudomonas aeruginosa* associated with negative interactions on coliform bacteria growth. *Canadian Journal of Pure and Applied Sciences*, 4: 1133–1139.

Viana A. A. G., Oliveira B. T. M., Cavalcanti T. G., Sousa K. A., Mendonça E. A. M., Amaral I. P. G., et al. (2018). Correlation between pyocyanin production and hydrocabonoclactic activity in nine strains of *Pseudomonas aeruginosa. International Journal of Advanced Engineering Research and Science*, 2018(5): 212–223.

Vipin C., Ashwini P., Kavya A. V., & Rekha P.D. (2017). Overproduction of pyocyanin in *Pseudomonas aeruginosa* by supplementation of pathway precursor shikimic acid and evaluation of its activity. *Research Journal of Pharmacy and Technology*, 10: 533–536.

Wang X., Cai W., Gerrits van den Ende A. H. G., Zhang J., Xie T., Xi L., et al. (2018). Indoor wet cells as a habitat for melanized fungi, opportunistic pathogens on humans and other vertebrates. *Scientific Reports*, 8: 7685, doi: 10.1038/s41598-018-26071-7.

Wei S., Jiang Z., Liu H., Zhou D., & Sanchez-Silva M. (2013). Microbiologically induced deterioration of concrete: a review. *Brazilian Journal of Microbiology*, 44(4): 1001-1007.

Wongsagonsup R., Shobsngob S., Oonkhanond B., & Varavinit S. (2005). Zeta potential (ζ) analysis for the determination of protein content in rice flour. *Starch*, 57(1): 25-31.

Yin W., Wang Y., Liu L., & He J. (2019). Biofilms: the microbial "protective clothing" in extreme environments. *International Journal of Molecular Science*, 20(14): 3423, doi: 10.3390/ijms20143423.

Zock J-P., Jarvis D., Luczynska C., Sunyer J., & Burney P. (2002). Housing characteristics reported mold exposure, and asthma in the European Community Respiratory Health Survey. *Journal of Allergy and Clinical Immunology*, 110(2): 285–292.

Żukiewicz-Sobczak W.A. (2013). The role of fungi in allergic diseases. *Postępy Dermatologii i Alergologii*, 30(1): 42-45.

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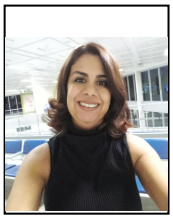

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