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: 6.64±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 °C. 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.

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 algacides (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 (Jamsâ 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 reconstituted 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 spectrophotometry 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 25°C (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 30°C 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 MICx2 wells to new microplates containing SDB 2%. After incubation at 30°C 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 1-3x10^6 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 cm². 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: 0.45±0.001) were observed. 0.176), zeta potential ($\zeta$) 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).

### TABLE I: CHARACTERISTICS OF NANOSYSTEMS OVER 90 DAYS

| Np-Pyo | Time (day) | pH (±0.01) | Size (±0.30 nm) | PDI (±0.10) | $\zeta$ (±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               | Blue, with two phases that homogenized under agitation |
|        | 21         | 6.18       | ---            | ---         | ---                 |                             |
|        | 30         | 6.18       | 581.90         | 0.58        | -5.18               |                             |
|        | 60         | 6.22       | 595.30         | 0.38        | -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) | $\zeta$ (±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               | White, with two phases that homogenized under agitation |
|        | 21         | 6.42       | ---            | ---         | ---                 |                             |
|        | 30         | 6.45       | 286.76         | 0.43        | -7.53               |                             |
|        | 60         | 6.41       | 249.53         | 0.38        | -5.85               |                             |
|        | 90         | 6.39       | 311.43         | 0.75        | -5.33               |                             |

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

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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 to be 160 MPN/100µL/cm² (control), 23x10³ MPN/100µL/cm² while A. niger was 360 MPN/100µL/cm² (control, >23x10³ MPN/100µL/cm²), 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 fungal 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).

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 encapsulation 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 (Gutierrez 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.

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.
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 & Rautema-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 & Deákóvá, 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 amenasalism 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/cm². 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

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

Aleksej I., Dragh I., B. M., Ritossa M., L. Gros, M., Oswald I. P., et al. (2017). Aerosolization of mycotoxins after growth of toxigenic 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., Patau J.-L., Stéb M.-J., et al. (2005). Self-assembly of an amphotrophic iron(III) chlorides: 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 exotoxins produced by Enterococcus 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 polypropyleneimide by catalytic solvent shifting: 1. Characterization and mechanisms. Macromolecules, 52(4): 1970-1980.

Barton S. S., & Rowe J. J. (1981). Antibiotic action of pyocyanin. Antimicrobial Agents and Chemistry, 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(1): 119-124.

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., & Burge A. (2015). Self-assembly of an amphotrophic iron(III) chlorides: mimicking iron acquisition in marine bacteria. Angewandte Chemie, 117(17): 2636-2638.

Cappitelli F., Cattò C., Birnbaum D. T., Kosmala J. D., & Burge A. (2015). Self-assembly of an amphotrophic iron(III) chlorides: mimicking iron acquisition in marine bacteria. Angewandte Chemie, 117(17): 2636-2638.

Cavalcanti F., Cattò C., & Birnbaum D. T. (2016). Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles. Biofouling, 26(1): 1-11.

Chevaliers T., Damon M., Johnson L. (2015). Slow release of a biocidal agent from polymeric microprecipitates for preventing biodeterioration. Progress in Organic Coatings, 76: 269-276.

Connelly J., Akkapeddi P., Yadav V., Manjunath G. B., Updu D. 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.

Dias S. B. D., Jaramillo L. Y. A., Guedes D., Duran R., Carbon A., Bertolino L. C., et al. (2016). Relationship between biofilm formation and antimicrobial resistance in Gram-negative bacteria. Microbial Drug Resistance, 25(1): 72-79.

Eisenhart R., Manton D., & Rosei F. (2015). Antibacterial coatings: Challenges, perspectives, and opportunities. Trends in Biotechnology, 33(11): 637-652.

ELS. (2002). Reference method for broth dilution antifungal activity. Approved standard M3-A. Wayne: CLSI.

Ferreira A., D’Alessio G., Marchi M., & D’Andrea R. (2000). 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.

Fernández L., López Y., Muñoz E., Roló D., Ardanuy C., Martí S., et al. (2019). Susceptibility testing of filamentous fungi. Antimicrobial Agents and Chemotherapy, 55(1): 173-177.

Garin M. A., Gómez-Alarcon G., Vizcaino C., & García M. T. (1993). Biochemical mechanisms of stone alteration carried out by filamentous fungi living in monuments. Biocorrosion, 19: 129-147.

Gonzalez J.A. (2009). Antimicrobial coatings: Challenges, perspectives, and opportunities. Trends in Biotechnology, 33(11): 637-652.

Gorham 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.

Hamlin P. J., Huisman A. F., Al-Mahdawi M. A., Alkerim N. F. A., & Alrhanan E. S. A. (2017). Bioactivity of pyocyanin of Pseudomonas aeruginosa clinical isolates: A variety of human pathogenic bacteria and fungi species. The International Arabian Journal of Antimicrobial Agents, 7: 2, doi:10.3823/8123812.

Jain S. S., Mahbub R., Holopainen U., Ropponen J., Savolainen J., & Rispens A. C. (2013). Slow release of a biocidal agent from polymeric microprecipitates for preventing biodeterioration. Progress in Organic Coatings, 76: 269-276.

Jameel Z. J., Hussain A. F., Al-Mahdawi M. A., Alkerim N. F. A., & Alrhanan E. S. A. (2017). Bioactivity of pyocyanin of Pseudomonas aeruginosa clinical isolates: A variety of human pathogenic bacteria and fungi species. The International Arabian Journal of Antimicrobial Agents, 7: 2, doi:10.3823/8123812.

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

Sánchez-Espinosa, K. C., Rojas-Flores T. I., Davydenko, S. R., Venero-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/s11356-021-14321-8.

Khare E., & Arora N. K. (2011). Dual activity of pyocyanin from Pseudomonas aeruginosa – Antibiotic against phytopathogenic bacteria and fungal species. The International Arabian Journal of Antimicrobial Agents, 7: 2, doi:10.3823/8123812.

Lekshmi U., Ramaswamy D., & Dharmaraj S. (2014). Pyocyanin: Production, applications, challenges and new insights. World Journal of Microbiology and Biotechnology, 30: 1159-1168.

Lorincz L. K., & Ledergerber B. (2002). Antimicrobial Chemotherapy, 54: 1019-1024.

Galgoczy-Martins E., Morillas H., Maguregui M., Padilla-Camello K., Marcadé I., Morgado-Gamo 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 Biodeterioration and Biodegradation, 147: 10847, doi: 10.1016/j.ibiod.2019.108474.

Lekshmi U., Ramaswamy D., & Dharmaraj S. (2014). Pyocyanin: Production, applications, challenges and new insights. World Journal of Microbiology and Biotechnology, 30: 1159-1168.
Palko M., Deáková K. (2014). Bioactivity of pyocyanin in Pseudomonas aeruginosa. Microorganisms, 2(1): 116.

Özyürek S. (2009). Microbial communities on painted wet and dry external surfaces of a historic fortress in Niterói, Brazil. Environ Sci Technol, 241(3): 232–239.

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.

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

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

Van Dijck P., Sijmens L., Bossuyt M., Trotschel C., et al. (2018). Correlation between pyocyanin production and hydrocyanogenic 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. (2019). 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.

Wongsapongsup R., Shobnag S., Onkhankon 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). House dust and occupational mold exposure, and asthma in the European Community Respiratory Health Survey. Journal of Allergy and Clinical Immunology, 110(2): 285–292.

Zukewicz-Sobczak W.A. (2013). The role of fungi in allergic diseases. Postepy Dermatol i Alergol, 30(1): 42-45.
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