Microbiological contamination of water-based paints from an industry in the state of Paraná, Brazil

Contaminação microbiológica de tintas à base d'água de uma indústria do estado do Paraná, Brasil

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Abstract

A paint company in the state of Paraná in Brazil had several batches of water-based acrylic paints contaminated with microorganisms. Three fungi and one bacterium were isolated. The bacterium was identified as *Stenotrophomonas maltophilia*. Three commercial fungicides (Fungicide I, II and III) and three bactericides (Bactericide I, II and III) were evaluated in attempts to prevent microbial growth. Fungicide II (1 g/L) was the most efficient fungicide of fungal isolates A and B, while Fungicide I was a better inhibitor of isolate C at the lowest concentrations studied. Bactericide II and III inhibited bacterial isolate at a concentration of 1 g/L, while 2 g/L of Bactericide I did not inhibited its growth. Inhibition tests were standardized and used to quantitatively evaluate the bactericides demonstrating that Bactericide III (2 g/L) inhibited 80 % of bacterial isolate.
Key words: Paint. Microbial contaminants. Bactericides. Fungicides. *Stenotrophomonas maltophilia*.

Resumo

Uma indústria de tintas do Estado do Paraná (Brasil) teve vários lotes de tintas acrílicas à base d'água contaminadas por microrganismos. Três fungos e uma bactéria foram isolados. A bactéria foi identificada como *Stenotrophomonas maltophilia*. Três fungicidas comerciais (Fungicida I, II e III) e três bactericidas (Bactericida I, II e III) foram avaliados quanto ao potencial de inibição do crescimento microbiano. O Fungicida II (1 g/L) foi o mais eficiente para os isolados fúngicos A e B, enquanto que o Fungicida I inibiu melhor o isolado C nas menores concentrações estudadas. Os Bactericidas II e III inibiram o isolado de bactéria quando na concentração de 1 g/L, enquanto que o Bactericida I (2 g/L) não inibiu o crescimento desta. Os testes de inibição foram padronizados e utilizados para avaliar quantitativamente os bactericidas, e demonstraram que o Bactericida III (2 g/L) inibiu 80 % o isolado de bactéria.
Palavras-chave: Tinta. Contaminantes microbianos. Bactericidas. Fungicidas. *Stenotrophomonas maltophilia*.

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Introduction

Water-based paints comprise inorganic and organic materials, and consequently are susceptible to microbial infections during the manufacturing stages of paint production and their storage as the packaged product. Exposure to various physical and chemical phenomena, such as high temperatures and humidity, also appears to facilitate microbial attack on paint (LUCCHESI, 2003). The effects of paint biodeterioration are easily detectable (Table 1) and water-based paints appear to be more susceptible to biodeterioration than oil paints (DEY et al., 2004).

Table 1. Effects of microbial degradation on paint ingredients (DEY et al., 2004).

Components	**Consequence of microbial action**
cellulosic thickeners	loss of viscosity, gas production, pH shift
dispersants	colour shift, precipitation of pigments, gelling
coalescing agents	gloss reduction, poor freeze-thaw stability, chalking, porous film, poor adhesion
Antifoam	foaming, porous film
Dispersed colour	off-colour, uneven colour, agglomeration of colorant

Microbial contamination of paint can occur during its manufacture, with the majority of the contaminants being present in the source of water, in the raw materials and also in equipment vessels and plumbing lines (tanks and pipes) (FAZENDA, 1995). Certain microorganisms produce biofilms that can adhere to the surfaces of plumbing systems containing water, processing tanks and other environments at the factory, and these in general, are very resistant to the treatment with biocides and deodorants (WINKOWSKI, 1999).

Biodeterioration of paints by fungal and bacterial agents present in the packaged product and after painting has been described in the literature (ARQUIAGA; CANTER; ROBERTSON, 1995; BJURMAN, 1999; DORNIEDEN; GORBUSHINA; KRUMBEIN, 2000). The peculiar chemical characteristics of paints themselves (presence of polyamides, epoxy resins, chlorides and even some organic solvents and water) can serve as sources of nutrients for the contaminating microorganisms (STRANGER-JOHANNESSEN; NORGAARD, 1991). Among the bacteria associated with the deterioration of paints present in the packaged product, species of *Pseudomonas*, *Flavobacterium*, *Escherichia* and *Bacillus* have been identified and described for degrading and modifying the characteristics of paints (FAZENDA, 1995).

Biocides constitute a heterogeneous group of chemical agents with diverse mechanisms of antimicrobial action; microbiostatic (inhibiting microbial growth) and microbiocidal (causing irreversible or irreparable damage to the microbial cell) effects (DENYER, 1990; EUCHI, 2002). The biocides generally act during the vegetative stage of growth of the microbial cell, as the resistance to biocides develops during the period of sporulation (RUSSELL, 1990).

Changes in the active toxicity principle of biocides have seen the substitution of highly toxic compounds (pentachlorophenol and mercury used during the 1960's and 1970's, and organic composites such as formaldehydes and phenols in the 1980's) for more favourable compounds such as isothiazolinones that are less toxic and aggressive in the environment, (MATTEUCCI, 1989). However, these biocides still do not offer the same efficacy as mercuric compounds in the adequate preservation of paints and paintings (LEITE; MURRO, 1999). Microbial resistance to the action of these biocides has been related to the resistance to antibiotics (RUSSELL, 1990), and this is related to the

production of β-lactamases capable of hydrolyzing the β-lactams commonly present in antimicrobial agents including antibiotics (KATAOKA, 2003).

The objective of the work presented herein was to isolate and characterize the microorganisms from different batches of water-based paints from a paint company in the state of Paraná (Brazil), and to determine the potential biocide efficacy of some commercial bactericides[6] (Bactericide I, a composite of hemiacetals and isothiazolinones; Bactericide II, a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; Bactericide III, a mixture of dimethylurea, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) and fungicides (Fungicide I, a composite of heterocyclic compounds, isothiazolinones and benzimidazol; Fungicide II, 2-N-octyl-4-isothiazolin-3-one; Fungicide III, 2-methoxycarbamoylbenzimidazole;), and their use during the manufacturing process of paint in attempts to control microbiological contamination and activity.

Material and methods

Microbial isolation techniques

Paint samples were obtained in triplicate from some identified phases of the industrial process considered as critical points of microbial contamination: reservoirs, pipes and tubing, tanks and also some raw materials, such as antifoam, thickeners, dispersants and emulsions, as well as water, and paints of contaminated batches. The samples were transferred to potato-dextrose agar (PDA) plates to allow development of microbial colonies. Samples collected *in-situ* in the contaminated places were carried out using the swab technique (GUGLIELMINETTI et al., 1994). In the laboratory, the collected material was transferred to PDA plates and left to grow at 28 °C, and colonies isolated and subcultured until pure as observed by homogeneous growth. The purified isolates were maintained at 4 ºC on PDA slants, and subcultured at intervals of 3 months.

For identification of bacteria, the samples were transferred to blood agar (BA) plates to allow development of microbial colonies. Then, the colonies were submitted to the Gram stain, and it was observed that all the colonies were Gram-negative. These colonies were next transferred to MacConkey agar (MC), and those that grew were then submitted to the following biochemical tests: oxidase, motility and oxidation-fermentation (OF). As all of the bacteria tested were oxidative, these were then identified by the API 20 NE system (bioMérieux, Marcy-L' Etoile, France).

Evaluation of commercial biocides by the pour-plate method

Inoculum was prepared by growing the isolated microbial strains on PDA at 28 °C for 5 days, and using agar-plugs of 5 mm diameter colonized with cells. Four plugs were transferred to assay tubes containing 10 mL isotonic saline solution (9 g/L). The tests were carried out in triplicate, and samples containing fungal mycelium, fungal spores or bacterial cells from each purified isolate, were mixed in PDA before the agar solidified. Inhibition tests were carried using sterilized filter paper discs of 5 mm diameter placed within the centre of each PDA plate. To each disc, 8 µl of an aqueous solution of each fungicide (Fungicide I, Fungicide II and Fungicide III) or bactericide (Bactericide I, Bactericide II and Bactericide III) was added at a concentration of 1 g/L (recommended by the manufacturer); 2 g/L (as used by the industry) and 8 g/L (chosen experimentally as excess of biocide). The plates were incubated at 28 °C for 7 days and the halo of inhibition observed at intervals of 24 h. Radial growth was measured as colony diameters (cm) at 4-equidistant points taken from the centre

[6] As we didn't have permission to divulge the names of the commercial biocides used in this study, we adopted the acronyms Bactericides I, II and III, and Fungicides I, II, and III to refer to these biocides.

of the growing fungal colony, and the average value taken. All experiments were carried out in triplicate and the reported data represents average values ±SD.

Spectrophotometric evaluation of commercial biocides

The bacterial isolate were grown in 125 mL Erlenmeyer flasks on glucose (10 g/L) containing 25 mL liquid culture medium according to Collins and Lyene (1989), and in the presence of the biocides (2 g/L). After inoculation, the flasks were incubated at 28 °C for 48 h under shaking conditions (180 rpm). The bacterial culture solution was diluted in sterile water (1:10) and scanned within the spectral range of 400 to 800 nm to monitor the absorbance to develop the inhibition tests.

Results and discussion

The microorganisms isolated from industrial water samples used in this work for the inhibition tests are shown in Table 2. The fungal strains have not been identified, being differentiated in accordance with their visual characteristics and named as isolates A, B and C. The isolate of bacteria was identified as *Stenotrophomonas maltophilia* (bacterial isolate).

Table 2. Nature of the microbial contaminants isolated from industry water samples.

Microorganisms	Characteristics
Fungal isolate A	filamentous white fungus
Fungal isolate B	filamentous green fungus
Fungal isolate C	filamentous light-yellow fungus
Bacterial isolate	*Stenotrophomonas maltophilia*

The microbial contamination of paints containing fungicides in their formulation is not uncommon, and has been described (GUGLIELMINETTI et al., 1994; BJURMAN, 1999). Inoue and Koyano (1991) isolated different fungal species from oil paints containing biocides based on pentachlorophenol and benzimidazol components, which included genera of the following fungi: *Cladosporium*, *Trichoderma*, *Rhizopus*, *Aspergillus*, *Alternaria*, *Penicillium* and *Fusarium*, as well as a several bacterial species. The isothiazolinone compounds, present in the majority of commercial fungicides currently used in paints, have been described as inhibiting the respiratory chain and the production of metabolic energy (DENYER, 1990).

The commercial fungicides evaluated in the inhibition tests included Fungicide I, Fungicide II and Fungicide III, separately, at concentrations of 1, 2 and 8 g/L (Figs 1A and B). Fungicide II (1 g/L) inhibited fungal isolates A and B more efficiently than Fungicides I and III at 48 h. After this period, the inhibition halo observed in the presence of Fungicides I and III with fungal isolate B decreased suggesting that by then the microorganism had acquired resistance to these biocides.

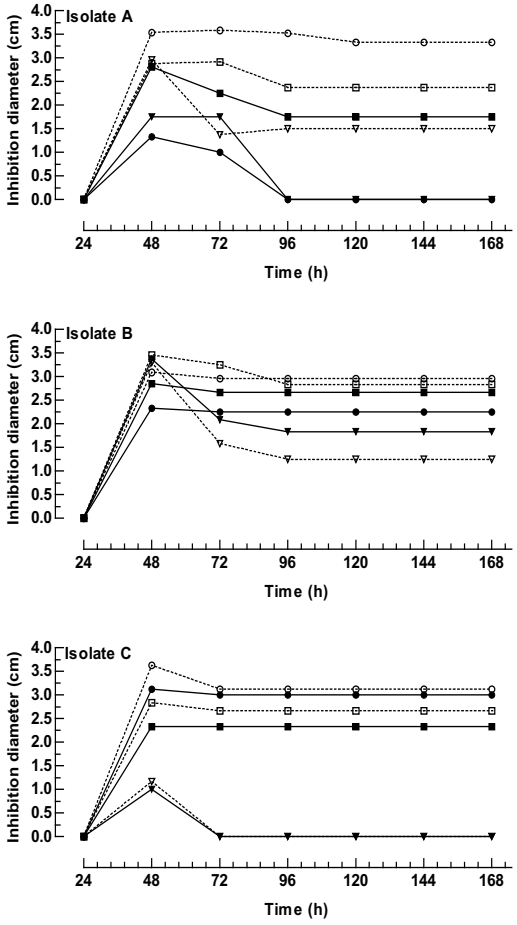

Figure 1. Evaluation of growth inhibition of fungal isolates A, B and C by commercial fungicides: Fungicide I (•), Fungicide II (■) and Fungicide III (▼) at 1 g/L; and Fungicide I (○), Fungicide II (□) and Fungicide III (▽) at 2 g/L.

When the fungicides were evaluated at a concentration of 2 g/L, an inhibition halo was observed in I68 h with Fungicides I, II and III, which corresponded to halo diameters of 3.33, 2.37 and 1.50 cm, respectively, for fungal isolate A, and 2.95; 2.83 and 1.25 cm, respectively, for fungal isolate B. However, for fungal isolate C (Fig 1C), Fungicide I was the most efficient fungicide evaluated at concentrations of 1 and 2 g/L. In the case of biocide Fungicide III, the inhibition halo decreased after 48 h, suggesting that the microorganism had already acquired resistance to the presence of this compound.

The presence of excess concentrations (8 g/L) of the fungicides in the culture medium resulted in the inhibition of growth for all fungal isolates. It was observed that fungal isolate A was less susceptible to Fungicide III, while fungal isolates B and C, presented greater resistance to Fungicides II and I, respectively. However, this concentration is impracticable for routine industry use due to the high cost and also the risk of inducing resistance to microorganisms.

Shirakawa et al. (2002) verified that use of paints containing dimethylurea and carbamate as biocides did not hinder the growth of some fungal species such as *Alternaria*, *Epicoccum*, *Pestalotia* and *Aureobasidium*. These fungi have been described as producing exopolysaccharides (GOATLEY, 1968; SCHIMID et al., 2001; MISAKI et al., 1984; GIBBS; SERVIOUR, 1992), which participate in the process of microbial deterioration. The occurrence of biofilms in domestic and industrial water piping can result in loss of water quality, corrosion of the pipes and contamination of industrial processes and products, some with pathogens (MORTON; SURMAN, 1994).

Some bacterial species such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Bacillus* and *Alcaligenes* have also been described in the biodegradation of paints and result from water sources (ARQUIAGA; CANTER; ROBERTSON, 1995). The bacterial isolate of the present work was identified as *Stenotrophomonas maltophilia* (bacterial isolate). This Gram-negative bacterium was originally identified as belonging to the *Pseudomonas* species and later reclassified as *Xanthomonas maltophilia*. *S. maltophilia* has been found in water, soil, plants (BROOKS et al., 2004) but rarely in humans and animals (DALAMAGA et al., 2003). Through the use of molecular biology techniques other species of *Stenotrophomonas* have been described recently as *S. african*, *S. nitritireducens*, *S. acidaminiphila* and *S. rhizophila* (COENYE et al., 2004).

The commercial bactericides evaluated in the inhibition of growth of *S. maltophilia* were Bactericides I, II and III, separately, in the concentrations of 1, 2 and 8 g/L. According to Fig 2, Bactericide III (1 g/L) was more efficient than Bactericides II and I, and the latter did not inhibit bacterial growth at this concentration. In the inhibition tests using the bactericides at a concentration of 2 g/L, a small inhibition halo was observed in the presence of Bactericides II and III. Bactericide I did not inhibit the growth of bacterial isolate. When evaluated at a concentration of 8 g/L, the bactericides totally inhibited bacterial growth.

The "pour-plate" method was not sensitive for the detention of growth inhibition of *S. maltophilia* when evaluated at low concentrations of the bactericides used. A spectroscopic method was therefore developed to analyze the potential activity of these biocides. Fig 3 illustrates the comparison of absorption spectra (400 to 800 nm) of the *S. maltophilia* culture medium with the control flasks (containing sterile culture medium in the presence or absence of the evaluated commercial bactericides at 2 g/L). The sterile culture medium did not absorb at 450 nm, while at this wavelength, the *S. maltophilia* culture medium presented maximum absorbance at 0.388 for bacterial isolate culture medium, respectively.

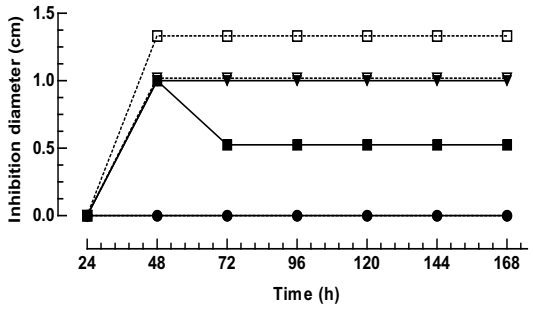

Figure 2. Evaluation of growth inhibition of bacterial isolate by commercial bactericides: Bactericide I (•), Bactericide II (■) and Bactericide III (▼) at 1 g/L; and Bactericide I (○), Bactericide II (□) and Bactericide III (▽) at 2 g/L.

In accordance with the evaluation of the scanned spectra, the wavelength selected for the inhibition tests was set as 500 nm, where maximum absorption of the bacterial culture medium and minimum absorption of the control flasks were observed, and thereby decreasing interference in the inhibition analysis by the spectroscopic method. The percentage of bacterial growth inhibition was scored taking into account the absorption of culture medium without the biocides. The maximum percentage (81 %) of inhibition scored for the growth of *S. maltophilia* was observed in the presence of Bactericide III (2 g/L). Using the absorption data, the scoring showed that Bactericides II and I (2 g/L) inhibited the growth of bacterial isolate by 32 and 76 %, respectively.

The results obtained for the spectroscopic method demonstrated that the "pour-plate" method was not suitable to evaluate the growth inhibition, as the "pour-plate" method could only evaluate the potential biocide at a concentration of 8 g/L, while by the spectroscopic method the efficacy of the bactericides was demonstrated at 2 g/L in developing bacterial growth.

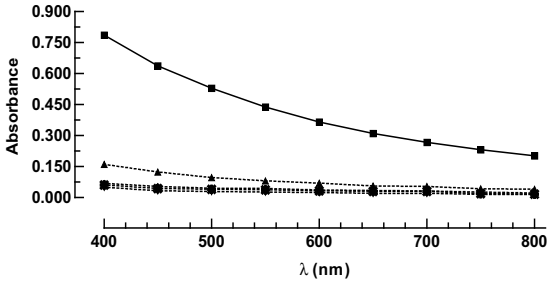

Figure 3. Absorption spectra of culture medium: control flasks (◆), bacterial isolate (■), Bactericide I (∗), Bactericide II (▼) and Bactericide III (▲).

Among the Gram-negative bacteria strains studied by Andrzejewska et al. (2004), *S. maltophilia* and *Bordetella bronchiseptica* were inhibited by high concentrations of benzimidazoles compounds (100 g/mL), which is the active principle used in

the composition of certain biocides. *S. maltophilia* has also been described for its capacity to degrade lactams and atrazine, besides presenting resistance to a diversity of antibiotics and xenobiotic composites (ROUSSEAUX; **HARTMANN; SOULAS**, 2001). The effect on the biodeterioration of paints through fungi and bacteria presents characteristics such as changes in viscosity, discoloration, appearance, development of gas and foul odour (DEY et al., 2004; TAN et al., 2008).

Isolation of microorganisms from critical points in the manufacturing process of paints at the factory and the evaluation of potential biocides of some commercial biocides has allowed the location of the origin and source of microbial contamination of the water-based paints, and to resolve this problem. It was observed that the biodeterioration of paint in the packaged product occurred due to contaminated water used as solvent, as the industry did not pretreat water sourced from artesian wells, and used in the process to make soluble resins. After the execution of this research work, the industry treated its reservoirs and equipment, and initiated a procedure of water treatment to be used in its process.

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