Natural products to control biofilm on painted surfaces

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Abstract

Purpose – This paper aims to study five vegetables extracts as possible additives to control bacterial growth on indoor waterborne paints. The extracts were obtained from the weeds Raphanus sativus, Rapistrum rugosum, Sinapis arvensis, Nicotiana longiflora and Dipsacus fullonum, used in traditional medicine as antimicrobial compounds.

Design/methodology/approach – Weeds extracts were characterized by Fourier transform infrared spectroscopy and UV–Vis spectrophotometry. Their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was also determined. Afterward, selected extracts were incorporated in waterborne paint formulations. The paints' antimicrobial activity was assessed against *S. aureus*, monitoring biofilm formation by environmental scanning electron microscopy.

Findings – As a general rule, results showed that tested paints were efficient in inhibiting biofilm formation, especially that formulated with Nicotiana longiflora.

Practical implications – The tested paints can be used to protect walls from microbial colonization, which shortened coatings' useful life by discoloration and/or degradation. Concomitantly, indoor microbial colonization by aerosols could be also diminished. This is especially important in places that should have high standards of environmental hygiene, as in the food industry, health-care and sanitary centers.

Originality/value – The main value of this research was to study the antimicrobial activity of weeds extracts and to incorporate them in waterborne paints to diminish bacterial biofilm formation. This biofilm discolors and degrades the paint, and causes health problems. The use of natural compounds in coatings is increasing because of the convenience of using renewable sources, such as natural antimicrobials, in paint formulations.

Keywords Bacteria, FTIR spectroscopy, Microorganisms, Biocides, Coating biodeterioration, Vegetables extracts, Antimicrobial paints, Biofilm, Inhibition

Paper type Research paper

The ability of microorganisms to colonize diverse surfaces, grow and develop into a biofilm constitute a serious problem for numerous industries including medical, food and marine (Videla and Herrera, 2005; Yebra *et al.*, 2006; Guerrero *et al.*, 2009; Héquet *et al.*, 2011). A biofilm can be defined as an accumulation of microorganisms and their extracellular matrix to form a structured community onto a given surface (Tenke *et al.*, 2004). These matrices protect microorganisms from being removed and facilitate their survival under unfavorable conditions or sudden climate changes (Otto, 2013). Moreover, it has been demonstrated that the biofilm shields bacteria from the action of antibacterial agents (Costerton *et al.*, 1995;

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Pigment & Resin Technology
47/2 (2018) 180–187

[ISSN 0369-9420]
[DOI 10.1108/PRT-01-2017-0004]

The authors are grateful to Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata and Comisión de Investigaciones Científicas de la Provincia de Buenos Aires for their sponsorship to do this research. The authors also wish to thank Dr Teresa Del Panno for her technical assistance.

Received 4 January 2017
Revised 5 April 2017
16 June 2017
Accepted 26 July 2017

Gilbert *et al.*, 2002). Several factors influence surfaces colonization by microorganisms, principally a high relative ambient humidity and/or temperature. The availability of organic compound such as cellulose, frequently present in a typical indoor paint as thickener, contributes to feed microorganisms (Garg *et al.*, 1995).

In recent years, substantial scientific evidence proved that environmental contamination of surfaces in hospital rooms plays an important role in the transmission of several key health care-associated pathogens (*Staphylococcus aureus*, *Enterococcus* species, *Clostridium difficile*, *Acinetobacter* species and norovirus; Costerton *et al.*, 1999; Weber *et al.*, 2013).

Cleaning or disinfecting the environment can reduce infection transmission (Kramer *et al.*, 2006). This fact has driven the control of microorganisms in the environment using new materials with inherent antimicrobial properties (Sambhy *et al.*, 2006; Schneider, 2013; Hoque *et al.*, 2015). In this sense, antimicrobial paints are very useful because they are designed to avoid bacterial and fungal colonization of the painted surface (Hare, 2000; Johns, 2003), thus contributing to the hygiene of buildings (Sontakke *et al.*, 2012) and extending the useful life of the coating. Taking into account that it is necessary to look for renewable and environmentally friendly antimicrobial agents, the development of ceiling and wall paints formulated with vegetables extracts with antimicrobial activity, instead of synthetic biocides, results promissory.

Vegetables extracts are a complex mixture of different compounds, generally related to each other, which may have antimicrobial activity on different targets (Inoue *et al.*, 2004; Laks, 1987; Scalbert, 1991). Among these compounds, polyphenols, flavonoids, terpenoids, alkaloids, etc. can be mentioned. Vegetables extracts as well as essential oils, both are gaining more acceptance among researchers because of their natural origin, abundance and safety of use (Binorkar and Jani, 2012; Bonjar, 2004).

The objective of this research was to evaluate five vegetables extracts to determine their ability to inhibit bacterial growth. The most active extracts were used to develop, later, an antimicrobial paint to prevent the microbial colonization of surfaces. Microbial colonization of surfaces is preceded by the biofilm formation. The extracts used were obtained from the weeds *Raphanus sativus, Rapistrum rugosum, Sinapis arvensis, Nicotiana longiflora* and *Dipsacus fullonum*, common in Argentina.

In a first step, the antimicrobial activity of the extracts was evaluated against two strains *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC, 6538). These bacteria are important because they are closely related to human health. For example, *S. aureus* strains, especially those resistant to the drug methicillin referred as methicillin-resistant (MRSA), play an important role in infections acquired by hospitalized patients. In a second step, water-based antimicrobial paints were formulated using the extracts, which exhibited antimicrobial activity. Finally, the resistance of waterborne paint against bacterial biofilm development was assessed. It was found that acrylic–styrene waterborne paints, containing *Nicotiana longiflora* inhibited biofilm formation.

2. Materials and methods

2.1 Selection of the vegetables

The weeds *Raphanus sativus* (Rs), *Rapistrum rugosum* (Rr), *Sinapis arvensis* (Sar), *Nicotiana longiflora* (Nl) and *Dipsacus fullonum* (Df), very common in the central part of Argentina, were selected as possible source of biocides. They were used previously in traditional medicine as antimicrobial compounds (Hanlon and Barnes, 2011; Pérez Gutiérrez and Pérez, 2004; Singh and Singh, 2013; Amel *et al.*, 2013; Al-Younis and Abdullah, 2009; Sezik *et al.*, 2001; Martinez *et al.*, 2004; Smith and Smith, 1942; Rosendal Jensen *et al.*, 1979).

As is seen in Table I, the weed extracts tested in this research consist of a mixture of many different compounds.

Rs extracts contain substances such as amino acids, polyphenolics and alkaloids (Hanlon and Barnes, 2011; Pérez Gutiérrez and Pérez, 2004; Singh and Singh, 2013).

Rr possess thiofunctionalized glucosinolates, which, according to bibliographic data, have some antimicrobial activity. The bioactivity is not attributed to intact glucosinolates but, instead, to products such as isothiocyanates, organic cyanides, oxazolidinethiones and ionic thiocyanate released upon enzymatic degradation by myrosinase (thioglucoside glucohydrolase in the presence of water) (Amel *et al.*, 2013).

The analysis of Sar extract confirmed the presence of classical antimicrobials such as phenols, tannins, flavonoids, alkaloids and saponins but glycosides, amino acids and carbohydrates, may be also found in the extracts. Phenolic extracts showed inhibitory effect on the growth of *Echerichia coli* and *Staphylococcus aureus* strains (Al-Younis and Abdullah, 2009).

Nicotine and other alkaloids are present in Nl (Martinez *et al.*, 2004; Smith and Smith, 1942), while phenolics and alkaloids are normally found in Df extracts, together with triterpenoids and iridoids (Zhao and Shi, 2011; Rosendal Jensen *et al.*, 1979).

2.2 Preparation of the extracts

The extracts from the selected weeds were obtained by refluxing the dried and crushed vegetables in methanol for 45 minutes. Afterward, the supernatant was filtered off and the solvent was eliminated under reduced pressure by rotary evaporation (Salazar *et al.*, 2011). The extracts were conserved in the fridge at 4°C.

2.3 Characterization of the extracts

Fourier transform infrared spectra (FTIR) were obtained to identify different functional groups present in the extracts. A Perkin–Elmer Spectrum One FTIR Spectrometer and the KBr pellet method were used.

The total soluble polyphenols (TP) content was determined by UV–Vis spectrophotometry using the Folin–Denis (FD) reagent (Ferreira *et al.*, 2004). The amount of TP is important because these compounds are referred to as antimicrobial agents (Laks, 1987; Scalbert, 1991).

FD reagent contains sodium molybdate and tungstate salts, which, in alkaline media, reacts with phenol groups forming intense blue colored complexes. A calibration curve was done with solutions of tannic acid as reference. The absorbance of

Table I Characteristics of the weeds used

Weed (Scientific name)	Argentinean common name	Common name in English	Origin	Area covered in Argentina	Main chemical constituents	References
Raphanus sativus	rábano, nabón	radish	East Mediterranean area	Whole country	Clucosinolates, acylated, anthocyanins, phenolics, isothiocyanates, methins, sapogenins, amonoacids, alkaloids	Hanlon and Barnes (2011), Pérez Gutiérrez and Pérez (2004); Singh and Singh (2013)
Rapistrum rugosum	Mostacilla	Annual bastard cabbage	Eurasia and Africa	Whole country	Phenols, tannins, flavonoids	Amel *et al.* (2013)
Sinapis arvensis	Mostaza de campo	Field mustard	Europe	Whole country	Phenols, glycosides, tannind, flavonoids, carbohydrates, alkaloids, aminoacids, saponins	Al-Younis and Abdullah (2009)
Nicotiana longiflora	Tabaco de campo, Sacha-tabaco	Longflower tobacco	South America	North and Central provinces	Nicotine, alcohols, terpenoids, fatty acids, sterols	Martinez *et al.* (2004), Smith and Smith. (1942)
Dipsacus fullonum	Cardencha, carda silvestre	Wild teasel	Eurasia and North Africa	Central provinces	Triterpenoids, iridoids, phenols, alcaloids	Rosendal Jensen *et al.* (1979), Zhao and Shi (2011)

the complex was measured at 750nm with a UV–Vis spectrophotometer (Spectrum–SP 2000 UV). The per cent of TP of each extract was calculated, with respect to the ctotal amount of extracted solids and, finally, expressed as per cent w/w.

2.4 Antibacterial activity of the extracts

Escherichia coli, ATCC 11229 (Gram-negative) and *Staphylococcus aureus*, ATCC 6538 (Gram-positive) were used to carry out the antibacterial activity tests. Strains were obtained from the collections of the University of La Plata.

The antibacterial activity was evaluated by the broth dilution method. The extracts containing the broth was serially diluted (1:1) in tubes (1000 to 62.5 ppm) that had been previously filled with 0.5 mL of a nutrient broth. At concentration higher than 1000 ppm, the extract solution becomes turbid and no determination could be done. The culture media was a conventional R3 broth (0.1 g yeast extract, 0.1 g protease peptone, 0.1g dextrose, 0.06g KH_2PO_4, 0.01g $MgSO_4$ and distilled water up to 100 mL). The growth and sterility control tubes contained no extracts. After the dilutions were completed, 0.5 mL of the diluted microorganism suspension (10^6 CFU/mL) was added to all except the sterility control tube. Bacterial suspensions were obtained from R3-agar cultures incubated for 24 h at 37°C. After the incubation period of 24 h at 37°C, the broths were examined for assessing bacterial growth. For each extract, the lowest concentration that inhibited microorganisms' growth was referred to as the minimum inhibitory concentration (MIC).

Recounts were done in plates with R3-agar by the drop method (the Miles–Misra Method) at the highest concentration tested (1000 ppm). Afterwards, the most active extracts were selected to be used in paint formulation (Liao *et al.*, 2011).

2.5 Paint formulation and elaboration

Paint preparation was done in a high speed disperser by mixing, in a first step, water with the different additives: dispersing, antifoaming and thickener. Then, the pigments (titanium dioxide, calcium carbonate) were added and finally the resin (an acrylic–styrene copolymer) and cosolvents were incorporated (Table II). The pigment volume concentration (volume of pigment/total volume of solids (pigment + resin), PVC) was 85.7; that recommended for ceiling paints (Stieg, 1973). Ceiling paints were selected because they are prone to biofilm development under normal indoor humidification cycles; principally in bathrooms and kitchens (Adam and Samson, 2011). Once the preparation was finished, the paint was filtered and kept under laboratory conditions until used.

The extracts Rr and Nl were incorporated into the original paint just before painting. These extracts were selected because they showed to be more active against the strains tested in the previous bioassay. The concentration of the biocides was 1 per cent by weight of the total paint composition. A paint without biocide was used as control.

2.6 Assessment of the capacity of biofilm inhibition by paints

The capacity of the experimental paints to inhibit bacterial colonization was assessed by environmental scanning electron microscopy (ESEM). *S. aureus*, a gram positive bacterium, was selected to do this test as it is considered one of the main causes of intrahospitals infections. Painted glasses, 0.7 × 1.2 cm, were immersed in 10 mL of culture media, with 10^6 CFU/mL of *S. aureus* and incubated for 10 days at 37°C. The culture media was replaced every day. After the incubation period, the samples were removed and washed three times with phosphate-buffered saline solution (PBS) to remove non-attached bacteria. Then, these panels were immersed in 2 per cent v/v

glutaraldehyde in PBS to preserve biological structures (Bellotti *et al.*, 2014). Finally, the panels were observed by ESEM under the conditions recommended by Stobie: 6 torr, 5°C, 15 KV (Stobie *et al.*, 2010).

3. Results

3.1 Characterization of the extracts

The TP content of the extracts, which is generally below 4 per cent w/w, was higher in the case of Df and Nl (Figure 1).

The FTIR spectra showed that all the extract had similar organic groups, but with different concentration, as the intensity of the bands were different (Figure 2). The band between 3,600-3,050cm^{-1} could be associated with OH groups present in aromatic or aliphatic alcohols. Df presented the most intense bands in this region. The bands which appeared at 2,980-2,920cm^{-1} correspond to the stretching of C–H bond in nitrogenous compounds such as alkaloids (Cooper, 1980; Baranska and Schulz, 2009). Nl, Rs and Sar had shown more intense response in this region. Carbonyl stretching frequencies were localized in the region between 1,700-1,600cm^{-1}. The position of these bands is affected by substituents in the carbons attached to the carbonyl group; specially the presence of an aromatic ring caused the bands to appear below 1700cm^{-1} (Cooper, 1980; Smith, 1999). The band around 1050cm^{-1} confirmed the presence of the alcohols groups, all the extracts presented strong absorption bands at this wavelength. The band at ~870cm^{-1} is because of C–H out-of-plane bending vibrations in aromatic rings; the more intense ones corresponding to Nl and Rr (Smith, 1999).

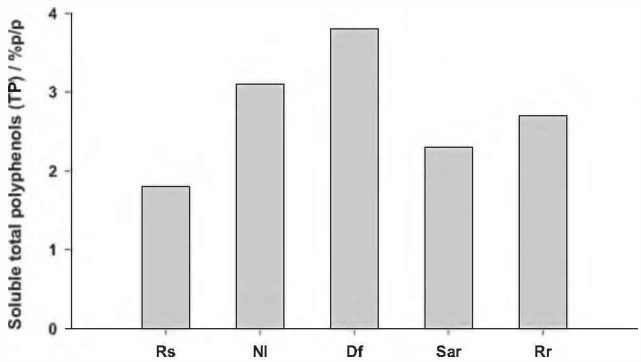
The presence of aldheydes was evidenced in the case of Df by its more intense reaction with the Tollens reactant.

3.2 Antibacterial activity of the extracts

The broth dilution method showed that none of the extracts completely inhibited bacterial growth at the tested concentrations. As a consequence, it was concluded that MIC was, in every case, higher than 1000 ppm. Bacterial recount was done in the tubes with the highest concentration (1000 ppm) and in the control tube. Results are shown in Figure 3, expressed as the ratio of $\log(CFU)_{extract}/\log(CFU)_{control}$. All the extracts proved to be efficient on inhibiting the growth *S. aureus* ($\log(CFU)_{extract}/\log(CFU)_{control} < 1$), especially in the case of Rr and Nl extracts. It must be highlighted that the bacterial growth diminished two orders of magnitude in the presence of Nl.

The antimicrobial action of the extracts toward *E. coli* inhibition was not relevant. The values of $\log(CFU)_{extract}/\log(CFU)_{control}$ were slightly less than or equal to 1.00 for all the extracts (Figure 3).

Figure 1 Soluble total polyphenols for each extract using Folin–Denis regent



Notes: Rs: Raphanus sativus; Rr: Rapistrum rugosum; Sar: Sinapis arvensis; Nl: Nicotiana longiflora; Df: Dipsacus fullonum

3.3 Selection of the extract to be included in paints formulation

Taking into account the results of the previous tests, the bioactivity of Rr and Nl could be partially attributed to the presence of polyphenols as their amount, determined by FD-reagent, was ~3 per cent w/w.

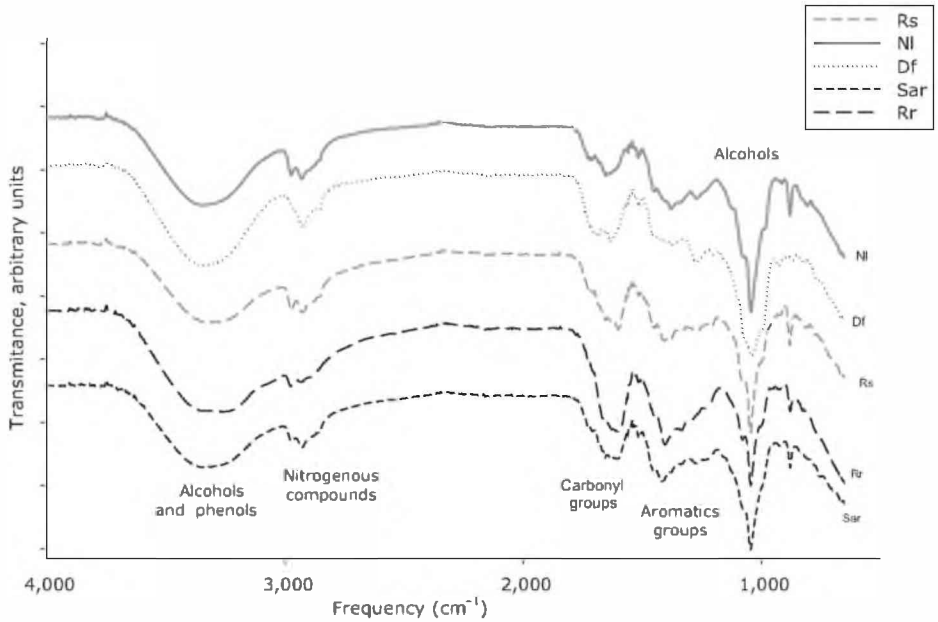
The poor antibacterial activity exhibited by Rs, Df and Sar would confirm that the only presence of polyphenols do not ensure bioactivity (Borrás-Linares *et al.*, 2015). It must be taken into account that the antimicrobial activity could be because of the presence of a specific phenollic compound and not to all of the polyphenols present in the extract. So, a high amount of TP not always implies an important antimicrobial activity (Borrás-Linares *et al.*, 2015).

Rr and Nl proved to be the most bioactive extracts against *S. aureus*; so, these extracts were selected to be incorporated in the formulation of antimicrobial paints.

Table II Control paint composition

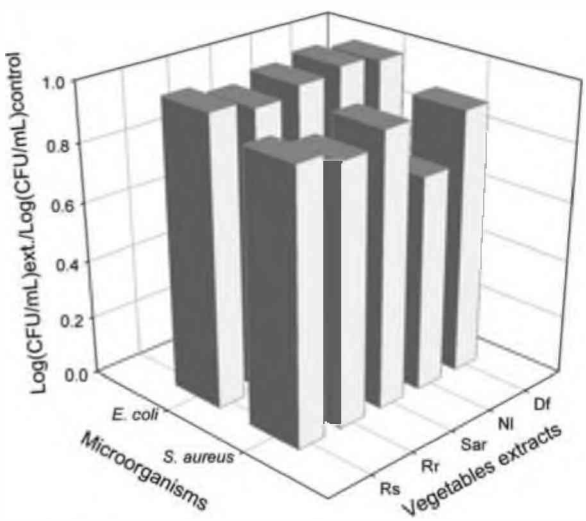
Component	% (w/w)	Brand
Distilled water	29.5	—
Antifoaming agent	0.26	Thyosil Q202, Diransa
Celullosic thickener	0.5	Cellosize 52000, Dow Chemical
Dispersant agents	0.39	Poliacril D40, Diransa
Wetting agent	0.13	AMP-95, Dow Chemical
TiO_2	19.0	Zamudio
Natural $CaCO_3$	41.6	Mica argentina
Precipitated $CaCO_3$	3.7	Mica argentina
Resin	7.2	Thyosil E 190, Diransa
Cosolvents	1.9	Butyl glycol and white spirit, Química Martin

Figure 2 FTIR spectra of the tested extract



Notes: Rs: Raphanus sativus, Rr: Rapistrum rugosum, Sar: Sinapis arvensis. Nl: Nicotiana longiflora, Df: Dipsacus fullonum

Figure 3 Relative growth as logarithm (CFU/mL) extract/logarithm (CFU/mL) control, obtained from the antibacterial activity tests of *E. coli* and *S. aureus*



Notes: Rs: Raphanus sativus, Rr: Rapistrum rugosum, Sar: Sinapis arvensis, Nl: Nicotiana longiflora, Df: Dipsacus fullonum

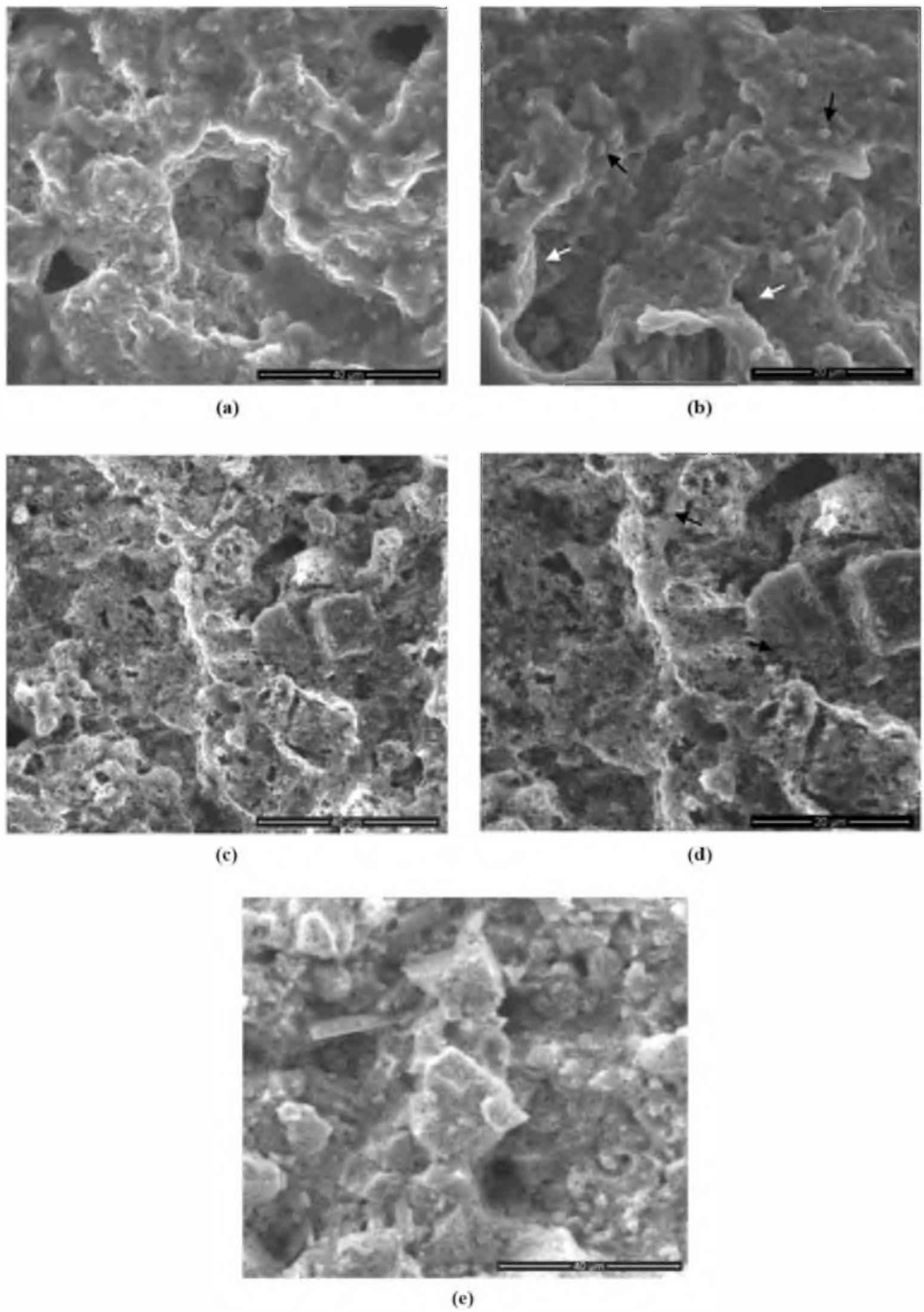
3.4 Assessment of the capacity of biofilm inhibition by paints

In Figure (4a) and (4b), it is clearly seen that bacteria colonized the whole surface of the control paint (without biocide). A thick biofilm with abundant extracellular material (white arrows) had completely covered the coating surface. Moreover, many bacteria emerging from the extracellular matrix could be seen; some of them were marked with black arrows (Figure 4b). These facts evidenced an active cell growth on the control coating. In general, it can be said that the tested paints were efficient in inhibiting biofilm formation, especially that formulated with Nl (Figure 4c and d). In this case, micrographs revealed a coating surface without biofilm formed on it, similar to that shown by the painted panel without inoculation (Figure 4e). Only some isolated bacteria may be seen (Figure 4d; black arrows). These results are in accordance with those obtained in the test to determine the antibacterial activity in which Nl resulted more efficient to control *S. aureus* growth. Paint with Rr had lower inhibitive action; the biofilm formation was incipient but not as important as in the control paint.

4. Conclusions

- The selected extracts showed no significant antimicrobial action against *E. coli*, but, in change, they appeared to be selective on controlling the growth of *S. Aureus*.
- The antimicrobial action seemed not to be related to the total polyphenols content. Other compounds present in the extracts could be responsible of their antimicrobial action.
- MIC was, in every case, higher than 1,000 ppm. Bacterial growth diminished, at least, two orders of magnitude in the case of *Nicotiana longiflora*.
- The acrylic–styrene waterborne paint containing *N. longiflora* extract was the most effective one on inhibiting *S. aureus* biofilm formation. The inhibition of biofilm growth became perceivable even at concentrations of ~1 per cent of the extract.

Figure 4 Micrographs (15 kV) obtained by ESEM of: (a) and (b) control paints (without biocides), the black and white arrows show extracellular matrix-embedded bacteria and abundant extracellular material respectively; (c) and (d) paint with Nicotiana Longiflora extract, the black arrows show some isolated bacteria; (e) control paint not inoculated



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