J. Mater. Environ. Sci. 6 (3) (2015) 749-755
ISSN : 2028-2508
CODEN: JMESCN

Comparison of the evolution of physico-chemical properties due to the single and combined adhesion of two species of the *Penicillium* genus on cedar wood.

H. Barkai[1*], M. Sadiki[1], S. El Abed[1,2], M. Moustakhim[1], M. Iraqui Houssaini[1], S. Ibnsouda Koraichi[1,2]

[1]*Laboratoire de Biotechnologie Microbienne, Faculté des Sciences et Techniques de Fès, Maroc.*
[2]*Centre Universitaire Régional d'Interface. Université Sidi Mohamed Ben Abdellah, Fès. Maroc.*

Received 24 Mai 2014, Revised 21 Nov 2014, Accepted 21 Nov 2014
[*]*Corresponding author: E-mail: ibnsouda@hotmail.com*

Abstract

In this study, we investigated firstly the evolution of physicochemical properties before and after the spores adhesion of *Penicillium expansum* and *Penicillium commun* on the surface of cedar wood. Secondly, we also observed the variation of the physicochemical properties after the dual adhesion of these two fungal strains. Thus, we found that the cedar was initially hydrophobic (θw =118.5° ΔGiwi = -6.09 mJ/m^2) with a significantly higher electron donor character (γ^- = 31.15 mJ/m^2) than the electron acceptor (γ^+ = 8.75 mJ/m^2). Then these surface properties of cedar were significantly changed following the adhesion of spores according to the contact time and depending on the *Penicillium* strains. The values for the physicochemical characteristics were (θw = 87.8°; ΔGiwi = -93.46 mJ/m^2 γ^- = 0.06 mJ/m^2 and γ^+ = 0.57 mJ/m^2) after 24 hours of incubation with the spores of *P. expansum* and (θw = 96 °; ΔGiwi = -46.18 mJ/m^2; γ^- = 4.09 mJ/m^2 and γ^+ = 4.40 mJ/m^2) with those of *P. commun*. However, although hydrophobicity is still maintained after the combined spore adhesion (*P. commun* + *P. expansum*), the latter allowed to find a significant increase of Lewis acid/base parameters compared to the single spore adhesions.

Keywords: Physico-chemical properties, Adhesion, *Penicillium*, Cedar wood, fungi, combination of strains.

Introduction

For several years, many studies have been conducted on the biofilms formation. They were examined at all stages of their formation, at small or large scale, which are developed by bacteria or fungi on different materials (wood [1], stainless steel [2, 3], silicone [4-6]…etc).

During biofilm formation, the initial step of the adhesion is considered as the important and most decisive of all. And it is divided in two steps: an adherence corresponding to a reversible adsorption of the microorganisms on the surfaces and a properly adhesion which correspond to their final fixation.

The adhesion phenomenon is subjected to the effect of several interactions such as Van der Waals forces, electrostatic forces and physico-chemical interactions (acid/base of Lewis: electron donor/acceptor characters, hydrophobicity/hydrophylicity and surface free energy). [3, 7-10].

These interactions are depending, at the same time, on the surface properties of the material studied and those of microorganisms adhering to the substrate [8]. Thus, the aim of our present study is to investigate the physicochemical characteristic of the cedar wood before and after adhesion of fungi, then to evoluate the change of these properties caused by the individual adhesion of two fungi strains: *Penicillium expansum* and *Penicillium commun*. Finaly, to compare the impact of the same physico-chemical characteristics of wood surface with the combination of the two strains studied.

2. Materials and methods

2.1. Preparation of the cedar wood surface

The substrate used in our study was the cedar wood (*Cedrus atlantica*) which is widely used in the construction of houses in the old medina of Fez. The cedar wood was cut into pieces which had the following dimensions: length = 3 cm, thickness = 0.4 cm and width = 1 cm. The roughness of the wood pieces was set in a range from 0.8 to 1µm by using a rugosimeter (Model : Mitutoyo Sj 301). Then, each piece of wood was washed six times with distilled water and then autoclaved at 120 ° C for 15 min.

2.2. Microorganisms, growth conditions and harvesting spores

Two strains of the *Penicillium* genus *(P. expansum and P. commun)* were isolated from cedar wood decay and identified in our laboratory [11]. Their growth was obtained at 25 °C using Malt Extract Agar. After 7 days of incubation, their spores were then harvested by scraping the culture surface in KNO_3 (0.1 M). The spore suspension was concentrated by centrifugation at 10,000 g for 15 min at 4 °C until a concentration of 10^7-10^8 spores / ml (counted with a hemacytometer).

2.3. Measurements of contact angles and calculation of the physicochemical characteristics

The surface properties of the cedar wood were characterized by the sessile drop technique [12,13]. Three measurements of contact angles were made on each wood samples using two polar liquids (water and formamide) and one apolar liquid (diiodomethane) with known energy characteristics (Table 1). All parameters of the physico-chemical characteristics of the surface (The surface free energy (ΔGiwi) of substrates, the Lifshitz-van der Waals components (γ^{LW}), the electron donor or Lewis base (γ^-) and electron acceptor or Lewis acid (γ^+)) were calculated by the equation of Young-Van Oss [14, 15]:

$$\gamma_L(\mathrm{Cos}\theta + 1) = 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + 2(\gamma_S^+\gamma_L^-)^{1/2} + 2(\gamma_S^-\gamma_L^+)^{1/2} \quad (1)$$

Where the terms (S) and (L) denote solid surface and liquid phases respectively.

The Lewis acid-base component was obtained by:

$$\gamma_S^{AB} = 2(\gamma_S^-\gamma_S^+)^{1/2} \quad (2)$$

And the samples degree of hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss and al. [14]. According to this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material when immersed in water (w): ΔGiwi. This latter was evaluated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta\mathrm{Giwi} = -2\gamma_{iw} = -2\left[((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + 2\left((\gamma_i^+\gamma_i^-)^{\frac{1}{2}} + (\gamma_w^+\gamma_w^-)^{\frac{1}{2}} - (\gamma_i^+\gamma_w^-)^{\frac{1}{2}} - (\gamma_w^+\gamma_i^-)^{\frac{1}{2}}\right)\right] \quad (3)$$

Table 1: Surface tension properties of pure liquids used to measure contact angles [15,16]

Liquids	γ^{LW} (mJ/m^2)	γ^+ (mJ/m^2)	γ^-(mJ/m^2)
Water (H2O)	21.8	25.5	25.5
Formamide (CH3NO)	39	2.3	39.6
Diiodomethane(CH2I2)	50.5	0	0

2.4. Adhesion of the spores on the cedar wood

The wood pieces were immersed in 10 ml of the KNO_3 suspension (0,1M) containing 10^7-10^8 spores / ml and incubated at 25 °C. Spore adhesion on the surface was carried out by sedimentation. After different contact

times indicated (2, 4, 6, 8, 10 and 24 hours), a piece of wood was removed and spores that had not adhered to the surface were rinsed three times with sterile distilled water, by moving slowly in a Petri dish [1, 16, 17]. Then the pieces were left for drying overnight at room temperature. Each sample was repeated three times.

3. Results and discussion

According to Vogler [17, 18] and Van Oss approach [1, 19, 20], when the value of the contact angle with water exceeds 65 °, the surfaces are characterized as hydrophobic and hydrophilic when the value of the contact angle is less than 65 °. Moreover a positive value of the free energy surface (ΔGiwi) means that the surface is hydrophilic and a negative value indicates that it is hydrophobic. The surface free energy gives a quantitative indication of the hydrophobicity of the substrate surface; while the contact angle with water permits a qualitative assessment of hydrophobicity.

Thus, according to this approach, the obtained results (Figure 1a and b) showed the hydrophobic character of the initial state of the studied cedar with a value of water contact angle greater than 65° (θw =118.5°) and a negative value of surface energy (ΔGiwi = -6.09 mJ/m^2). This is indicative of a qualitative and quantitative hydrophobicity. This hydrophobic character observed on the wood surface before spore adhesion corroborate the previous work of De Meijer et al. [21] and El Abed et al. [22] who noted a relative hydrophobicity of cedar (θw = 82.5 ° and ΔGiwi = -58.81mJ/m^2) and (θw = 69±2°; ΔGiwi < 0 mJ/m^2) respectively. For others wood species, Gérardin et al. [23] reported a significant hydrophilicity for pine and beech with the water contact angles of 55.4 ° and 54.5 ° respectively.

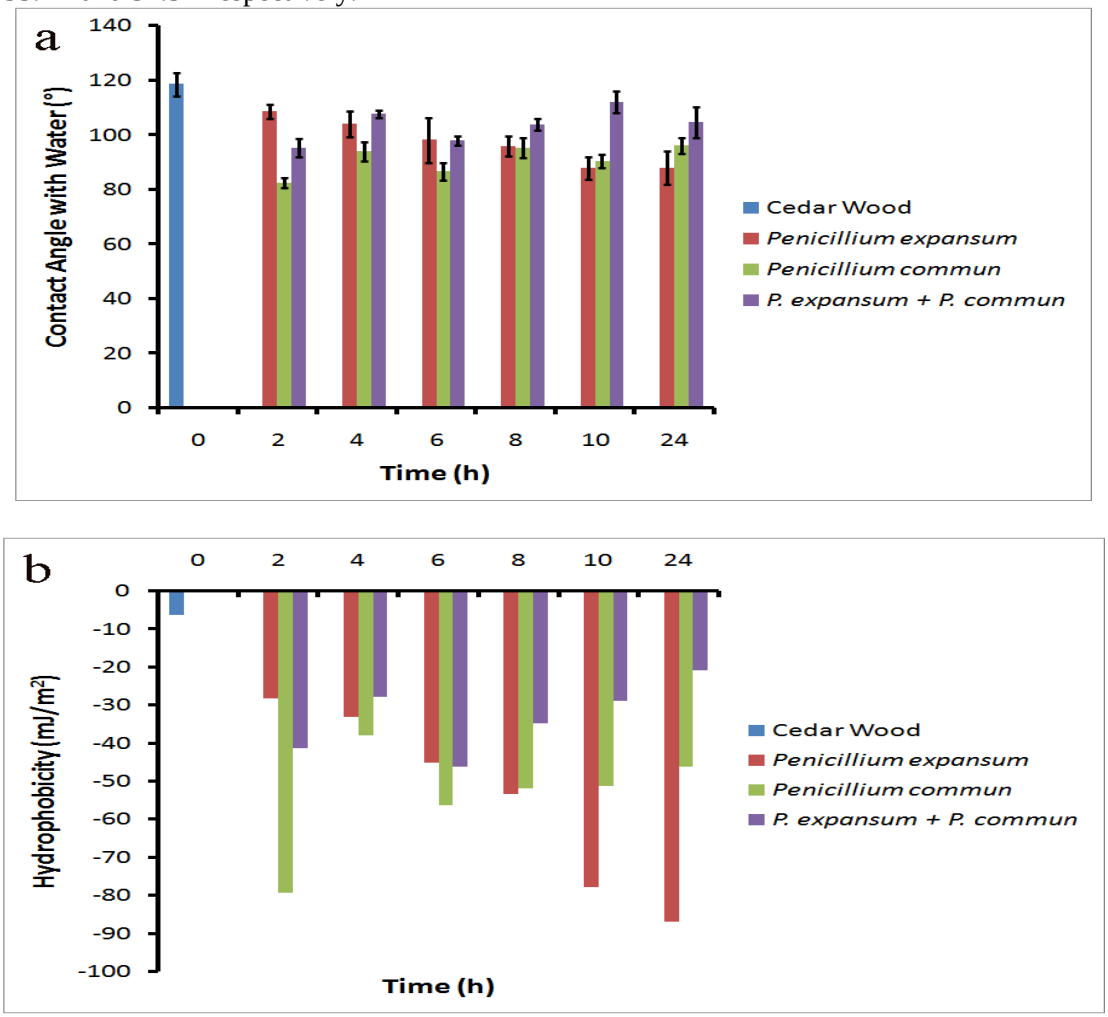

Figure 1: Variation of physico-chemical characteristics: contact angle with water (a) and the free surface energy (b) of the wood surface treated in function of the different contact times (2, 4, 6 , 8, 10 and 24 h) with the strains taken individually or in combination.

Figures 1 and 2 indicate the variation of physico-chemical characteristics (water contact angle, surface free energy, electron donor/acceptor characters) of the wood surface as a funtion of different contact times (2, 4, 6, 8, 10 and 24 h) with the strains taken individually or in combination.
So as it can be seen in Figure 1(a), compared to the initial state of cedar wood, the contact angles with water of the two strains indicate that the surfaces of the samples are hydrophobic with 96° and 87,8° after 24 h of incubation respectively in contact with *P. commun* and *P. expansum*. This qualitative hydrophobicity observed on surfaces of cedar pieces covered with fungal spores, is confirmed quantitatively by the analysis of the surface energies values shown in Figure 1 (b).

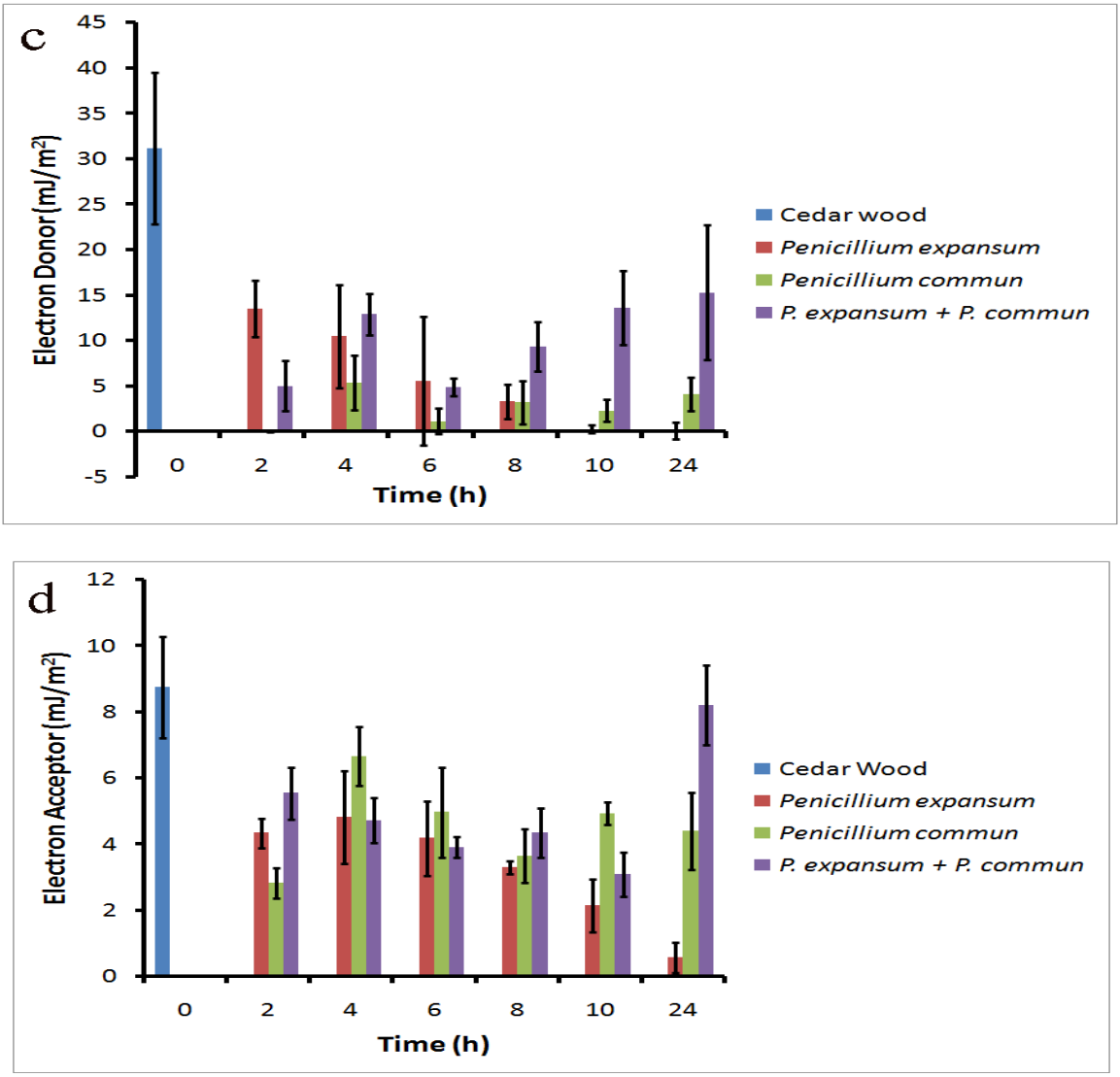

Figure 2: Variation of physico-chemical characteristics: Electron donor (c) and Electron acceptor (d) characters of the wood surface treated in function of the different contact times (2, 4, 6, 8, 10 and 24 h) with the strains taken individually or in combination.

Indeed, during the contact time between wood surface and our two fungi, the values of the surface energy remained negative at 24h with values of -46,18 mJ/m^2 and -93,46 mJ/m^2 respectively for *P. commun* and *P. expansum*. This decrease of hydrophobicity following the spore adhesion is predictable especially when we know that, unlike the cedar surface, our fungi spores are hydrophilic. El Abed [24] reported in its works, among others strains, that the surface of spores were hydrophilic: *P. expansum* (θw =45.3 °; ΔGiwi =15.2 mJ/m^2.), and *P. commun* (θw =17.9°; ΔGiwi = 8.5 mJ/m2). which would explain this decrease of hydrophobicity found.

Although it is generally accepted that microorganisms having hydrophobic properties tend to attach on hydrophobic surfaces; and inversely with hydrophylic microorganisms and hydrophilic surfaces; there are several other factors that can significantly influence the attachment of microorganisms to surfaces. these are, inter alia, the degree of surface roughness [25-28], the pH [29-31] or acid-base surface properties [7-10, 32, 33].
In this study, electron donor/acceptor characteristics of cedar surface have been highly modified after the adhesion of spores.
Indeed, the wood showed initially a significantly higher electron donor character (31.15 mJ / m^2) than the electron acceptor (8.75 mJ/m^2). However, these values declined sharply following the adhesion of the spores of the two strains on the surface of the wood. Thus, as can be seen on Figure 2 (a, b), the difference was significant from the first contact time (2 h) with a considerable decrease of the values of electron donor (4.09 and 0.06 mJ/m^2) and electron acceptor (4.40 and 0.57 mJ/m^2) characters after 24 h respectively for strains in the order mentioned above.
This significant decrease of the electron donor character as a function of time suggests its strong involvement in the adhesion of both *Penicillium* strains taken individually on cedar surface. This finding was consistent with the works of Van Oss et al. [34], Henriques et al. [35] and Hamadi et al. [32] who explained the importance of acid-base interactions in microbial adhesion on mineral surfaces. Mahdavi et al. [36] also reported the tendency of polymeric Biosurfaces to be donor electrons as is the case for our cedar samples.
As we have observed for hydrophobicity, the donor and electron acceptor characters also change when surfaces undergo a treatment. Indeed Gerardin et al. [23] reported that after heat treatment, pine and beech woods showed a slight modification of the electron acceptor component while the electron donor was strongly influenced. they also explained that these modifications were due to chemical changes that occur during the heat treatment. However, unlike our results where the polar component remains constant even after the adhesion of our both fungi spores when the dispersive component decreased significantly, Al-Turaif et al. [37] reported in their work that in the all cases of coating systems they studied, the dispersive components was higher than the polar components.
In addition to the analysis of the results of physicochemical properties of our two fungal strains taken individually and in particular of the physicochemical changes caused by their adhesion to the cedar surface, we also studied the evolution of these same surface properties when the adhesion is carried out with a mixture of spores of these two species of *Penicillium*.
In this second part of our study, it was found that the hydrophobic character of the samples surface was still maintained for the combination of spores (*P. expansum* + *P. commun*). However, it was also found that the hydrophobicity of combination was greater ($\theta w = 104.6°$; $\Delta Giwi = -20.95$ mJ/m^2) than both strains taken individually after 24 h.
Thus, as it can be seen in Figures 2 (c and d) we also find that the values of the electron donor and acceptor characters of the samples treated with spores combination were quite high ($\gamma^- = 15.29$ mJ/m^2, $\gamma^+ = 8.21$ mJ/m^2) at the end of the experiment (24 h) compared to those of the spores taken separately.
Indeed, it is rare, even impossible, to find a complete biofilm formed by a single microbial species in a natural ecosystem. Unfortunately, we did not found in littératture the similar cases to our study on the evolution of physicochemical characteristics of surface properties with combinations of strains. However, it is important to note the difference of the evolution of physicochemical parameters studied according to the single or dual adhesion of the *Penicillium* spores in time.
Indeed, when the results of the adhesion of both spores showed an important decrease the initial hydrophobicity as well as Lewis acid/base components over time, the results of the combination rather reveals a slight decrease compared to the control sample.
Furthermore, as the physicochemical surface properties help explain the phenomenon of adherence, several studies have also reported the ability of fungi to attach on substrates through hydrophobins which modify the properties of hydrophobic surfaces to hydrophilic surfaces and thus allow their adhesion. [38-41].
Other studies also report the use of essential oils to inhibit the growth of microorganisms in planktonic [42-44] or biofilm form [45-47].

Conclusion

In this study, we could clearly observe the impact of bioadhesion on physicochemical surface properties. The treatment of the cedar wood surface by the combination of spores also showed a significant change on the properties of the surfaces compared to bioadhesion of each spores taken individually. This could promote the participation of some microorganisms which do not have the ability to adhere to the substrates themselves.

References

1. Elabed S., Ibnsouda K.S., Houari A., Latrache H., *Mater. Sci. Eng. C*. 33 (2013) 1276.
2. Nguyen M.D., Le Duc M., Daisuke H., Duong V.H., Fumihiko T., Toshitaka U., *Food Cont.* 42 (2014) 94.
3. Nguyen H.D.N., Yang Y.S., Yuk H.G., *LWT - Food Sci. Techn.* 55 (2014) 383.
4. Depan D., Misra R.D.K., *Mater. Sci. Eng. C.* 34 (2014) 221.
5. Carolyn P.O.-D., Rodrigo C.S., Patrick J.A., *Inter. J. Pediatric Otorhinolaryngology*. 77 (2013) 223.
6. Xiang L., Peng L., Rathi S., Anindya B., Biswajit M., Suo H.L., Xiaodi S., Paul A.T., Susanna S.J.L., *Acta Biomaterialia* 10 (2014) 258.
7. Stefan R., Thomas B., Ralf J., *Dental Materials* 30 (2014) 702.
8. Habimana O., Semião A.J.C., Casey E., *J. Memb. Sci.* 454 (2014) 82.
9. Poncin-Epaillard F., Herry J.M., Marmey P., Legeay G., Debarnot D., Bellon-Fontaine M.N., *Mater. Sci. Eng. C.* 33 (2013) 1152.
10. Sampath T.S.K., DOI:10.1016/B978-0-12-415800-9.00002-4 (2013) 11.
11. Zyani M., Mortabit D., Mostakim M., Iraqui M., Haggoud A., Ettayebi M., Ibnsouda K.S., *Ann Microbiol.* 59 (2009) 1.
12. Blanco M.T., Blanco J., Sanchez-Benito R., Perez-Giraldo C., Moran F.J., Hurtado C., Gomez-Garcia A.C., *Microbios*. 89 (1997) 23.
13. Barkai H., El abed S., Sadiki M., Iraqui H.M., Ibnsouda K.S., *J. Adh.* (In press, Accepted Manuscript 2014).
14. Absolom D.R., Lamberti F.V., Policova Z., Zingg W., Van Oss C.J., Neumann A.W., *Appl. Environ. Microbiol.* 46 (1983) 90.
15. Sadiki M., Barkai H., Ibnsouda K.S., Elabed S., *J. Adh. Sci. Tech.*, 28 (2014) 1925-1934
16. Briandet R., Meylheuc T., Maher C., Bellon F.M., *Appl. Environ. Microbiol*. 65 (1999) 5328.
17. Hamadi F., Latrache H., Mabrrouki M., Elghmari A, Outzourhit A., Ellouali M., Chtaini A., *J. Adhes. Sci. Technol.* 19 (2005) 73.
18. Vogler E.A., *Adv. Colloid. Interface. Sci.* 74 (1998) 69.
19. Van Oss C.J., Chaudhury M.K., Good R.J., *Adv. Colloid. Interface. Sci.* 28 (1987) 35.
20. Van Oss C.J., Chaudhury M.K., Good R.J., *Colloid Interface Sci.* 128 (1989) 313.
21. De Meijer M., Haemers S., Cobben W., and Militz H., *Langmuir*, 16 (2000) 9352.
22. El abed S., Hamadi F., Latrache H., Iraqui M.H., Ibnsouda K.S., *Ann. Microbiol.* 60 (2010) 377.
23. Gérardin P., Petri M., Petrissans M., Lambert J., Ehrhrardt J.J., *Polymer. Degrad. Stability*. 92 (2007) 653.
24. El abed S., Mostakim M., Berguadi F., Latrache H., Houari A., Hamadi F., and Ibnsouda k. S., *Microbiology.* 80 (2011) 43.
25. Andre´a A.L., Ana L.M., Camila A.Z., Amanda F.W., Denise M.P.S., Carlos E.V., *Archoralbio*. 58 (2013) 1.
26. Bengourram J., Hamadi F., Mabrouki M., Kouider N., Zekraoui M., Ellouali M. and Latrache H., *Phys. Chem. News.* 47 (2009) 138.
27. Sousa C., Teixeira P. and Oliveira R., *Int. J. Biomat.* Article ID 718017, (2009) 9.
28. Kouider N., Hamadi F., Mallouki B., Bengourram J., Mabrouki M., Zekraoui M., Ellouali M. and Latrache H., *Int. J. Pure. App. Sci.* 4 (2010) 1.
29. El abed S., IbnsoudaK.S., Latrache H., Meftah H., Nezha J.T. & Hamadi F., *World J. Microbiol. Biotechnol.* 28 (2012) 1707.
30. Bong-Jae P., Abu-Lail N.I., *J. Colloid. Interface. Sci.* 358 (2011) 611.
31. Dao J., Fangqiong L., Guillermo M., Nicolas D., Eberhard M., Stephen A.B., Wen-Tso L., Thanh H.N., *Water Res.* 47 (2013) 2531.

32. Hamadi F., Latrache H., Zahir H., Elghmari A., Timinouni M., Ellouali M., *Braz. J. Microbiol.* 39 (2008) 10.
33. Karunakaran, E., Mukherjee, J., Ramalingam, B., Biggs, C.A., *App. Microbiol. Biotechnol.* 90 (2011) 1869.
34. Van Oss C.J., *Colloid. Surface A.* 78 (1993) 1.
35. Henriques M., Azeredo J. and Oliveira R., *Colloid. Surface B.*, 33 (2004) 235.
36. Mahdavi M., Jalali M. and Kasra R., *J. Biol. Sci.* 8 (2008) 502.
37. Al-Turaif H., Douglas W., Bousfield., *Progress. Organic. Coatings,* 49 (2004) 62.
38. Van Wetter M.A., Wösten H.A.B., Wessels J.G.H., *Mol. Microbiol.* 36 (2000) 201.
39. Wösten H.A.B., Schuren F.H.J., Wessels J.G.H., *EMBO. J.*, 13 (1994) 5848.
40. WESSELS J.G.H., *Mycologist.* 14, (2000) 153.
41. Markus B. L., *Curr. Op. Colloid. Interface. Sci.* 14 (2009) 356.
42. Ainane T., Askaoui Z., Elkouali M., Talbi M., Lahsasni S., Warad I., Ben Hadda T., *J. Mater. Environ. Sci.* 5 (2014) 2017.
43. Ayadi S., Abderrabba M., *J. Mater. Environ. Sci.* 5 (2014) 1872.
44. Laghchimi A., Znini M., Majidi L., Renucci F., El Harrak A., Costa J., *J. Mater. Environ. Sci.* 5 (2014) 1770.
45. SzczepanskiS. & LipskiA., *Food Cont.* 36 (2014) 224.
46. Stupar M., Grbić M.Lj., Džamić A., Unković N., Ristić M., Jelikić A., Vukojević J., *South African. J. Botany.* 93 (2014) 118.
47. Barbieri D.S.V., Tonial F., Lopez P.V.A., Sales Maia B.H.L.N., Santos G.D., Ribas M.O., Glienke C., Vicente V.A., *Archoralbio.* 59 (2014) 887.