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THE STUDY OF BACTERIAL GROWTH ON LOW-DENSITY POLYETHYLENE- LIGNIN COMPOSITE

Nawres N. jaber

Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah,Iraq

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ABSTRACT

In the present study, Low density polyethylene with various contents of lignin films were investigated for their resistance to bacterial adhesion. The disc diffusion method was employed for this purpose. The results of bacterial growth on low-density polyethylene (LDPE)–lignin composite were reported. lignin was added by 1%,2%,3%,4%,5%,10% and 15% of polyethylene weight. We compared initial adhesion and surface growth of *staphylococcus aureus , streptococcus pyogens, pseudomonus aerugenosa, Eschrishia coli and klebciala spp.* A 5 mm of test polymer composites were inoculated in the 9 cm Petri dish for 1-2 days and zone of inhibition and bacterial growing were observed and recorded. After 24 hours the bacterial growing of *S. aureus , S. pyogens and E. coli* were distributed all over the control agar_with no growth on the polymeric composites films while Both *P. aeruginosa* and *K. spp.* have a dense growing. After 48 hours a slight adhesion of *S. aureus* on polyethylene-lignin discs was found with radius of growing was 3 mm .For *P. aeruginosa* we have more dense growing . It was found that the lignin contents have no clear inhibition against tested bacteria where this might be due to a limitation of the agar disc diffusion method. The vanishing of inhibition zone was combined with the absence of adherent bacteria on the polyethylene films. More concentration of natural lignin is required to get inhibition effect.

INTRODUCTION

Polyethylene is the most widely used among thermoplastic, especially for packaging and constructions applications. In polyethylene packaging, microbial contaminations are of main concern. The application of antimicrobial agents into the polymer products is one of the methods to prevent the products from microbial contaminations [1]. There have been many antibacterial agents such as nisin, nano-silver,triclosan and sorbic acid anhydride that could be used by blending with polymers for inhibition of the bacteria growth, using conventional

polymer processing methods [2]. Some treatment methods for coating the anti-bacterial agents onto the polymer matrices may be required, depending on type, concentration and diffusability of bacteria through the matrices, and testing methods to evaluate the anti-bacterial performance [3].

In another hand microbial adhesion and biofilm formation on surfaces pose major problems and risks to human health. One way to circumvent this problem is to coat surfaces with materials that generates an abiotic surface with less bacterial attachment than uncoated surfaces. Hence a general bacteriocidal effect is not the reason for the antifouling effect. Where The formation of an infectious biofilm is initiated by transport and adhesion of microorganisms to the surface of the implant or device [4]. Initial microbial adhesion is extensively studied and generally believed to depend on the physico-chemical properties of the microbial and biomaterial surfaces [5].

After initial adhesion, surface growth of adhering microorganisms leads to the formation of a biofilm. Although this process is evidently significant in the pathogenesis of biomaterial-centered infections, there are only few studies on surface growth of adhering microorganisms, i.e. growth of those organisms in direct contact with the biomaterial surface [6].

Yet, these microorganisms play a pivotal role in biofilm formation as they link the entire biofilm to the biomaterial surface [7].Recently, Barton *et al.* [8] compared the initial surface growth rate of *Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli* on different orthopedic implant materials in a parallel plate flow chamber in whole growth medium. A correlation was found between the generation time of *P. aeruginosa* and the free energy of adhesion of the organisms for the different biomaterials. This correlation was not found for *S. epidermidis* and *E. coli*.

Habash *et al.* [9] studied adhesion of *P. aeruginosa* AK1 to silicone rubber in a parallel plate flow chamber from buffer and buffer, supplemented with 2 % nutrient broth. In broth supplemented buffer, a steady increase of the number of adhering organisms was found also after several hours, whereas in buffer stationary numbers of adhering bacteria were found after 1 h.

The aim of this study was to compare the initial adhesion and surface growth of five Testing bacteria on LDPE .

MATERIALS AND METHODS

The films were tested for the surface growth and the inhibition against the target bacteria by using an agar disc diffusion method (17)(When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar.

The properties of LDPE is shown in table (1) . Lignin was provided by chemistry department in polymer research center. The average lignin particle size used in this work was (< 125) μm. Figure (1) shows the lignin main moieties in atypical macromolecular assembly (11).Seven concentrations of lignin particles 1%, 2%, 3%, 4% , 5% ,10% and 15% wt% were used in the LDPE. Each film sample was cut in to a circle of 5 mm in diameter ,prior to being placed on an agar surface. A nutrient Agar was prepared and spared by 20 ml for each Petri dish. Followed by inoculation of 0.1 ml of suspension with optical density (OD=0.1) on 540 nm by spectrophotometer. Low density polyethylene discs are subject to attack more than one variety and species of microorganisms including (*s. aureus , s. pyogens, p. aerugenosa, E. coli and k. spp.)* where tested bacteria provided by microbiology laboratory in Veterinary College after insuring them by diagnoses tests (Gram stain test ,catalas test ,manitol test , urease, test , H_2S test , citrate test ,coagulase test ,indol ,mthyl red test and oxidase test) .All Petri dishes were left for almost 15to 30 minutes until dry and by that time all lignin modified LDPE discs are distributed in Petri dishes and kept in incubator under 37 C º for 24 hours .Followed by studying both bacterial adhesion and growth .

Table(1) properties of LDPE

Property	**LDPE**
Trade Name	**Scpilex (463)**
Density (g/cm^3)	**0.921-0.924**
Melt Index (g/10min)	**0.28-0.38**

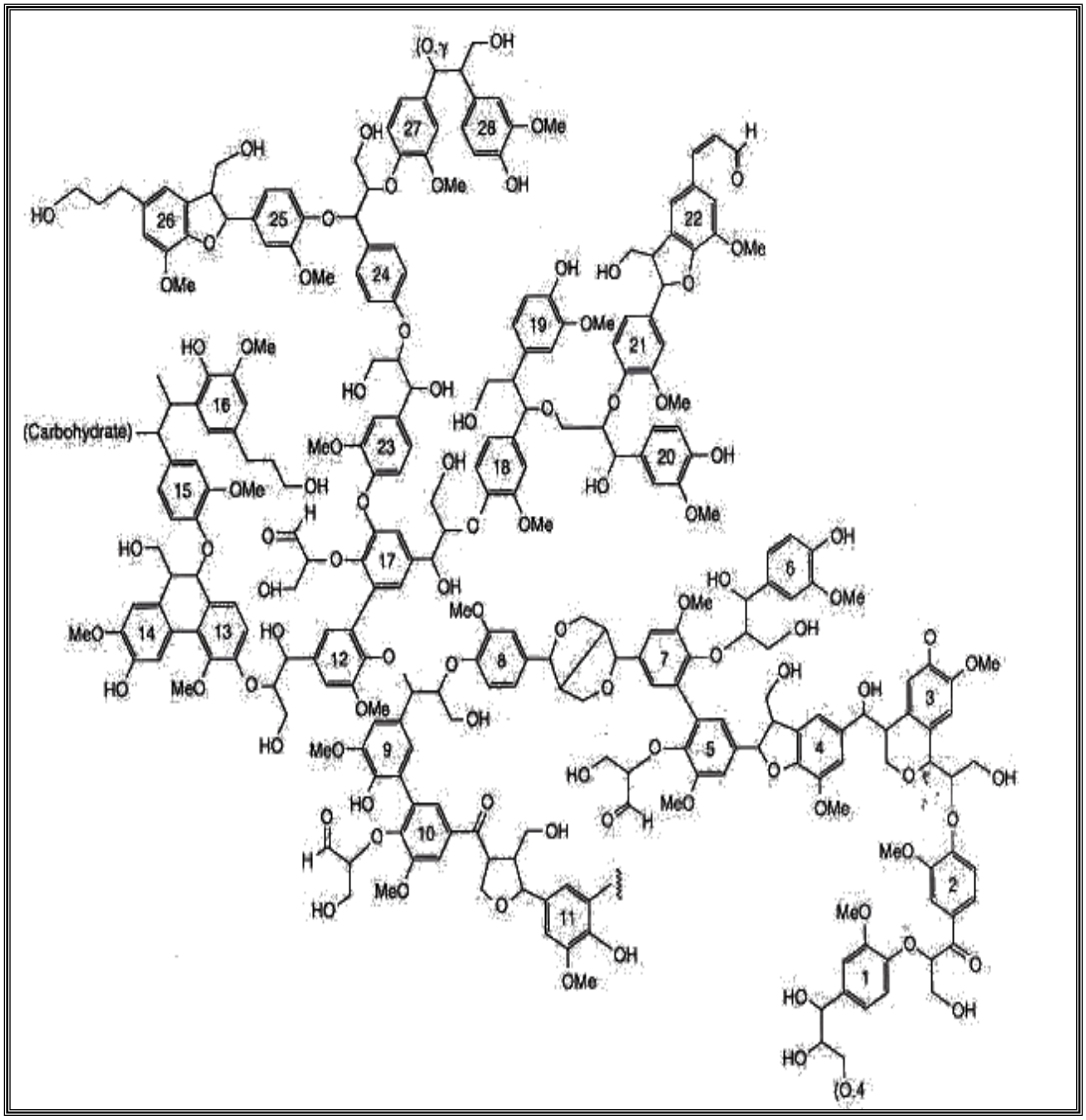


Figure (1) lignin structure [11]

RESULTS AND DISCUSSION

S. aureus S. pyogens bacteria and *E. coli* growing were distributed all over the control agar with no forming of a clear zone of inhibition around the film disc in the medium and the Colonies of *S. aureus S. pyogens bacteria* and *E. coli* could not be viewed in the clear zone directly above the film samples whereas such colonies were formed all over the Petri dish .Also the absence of growth on discs surface encouraging to use such kind of thermoplastic polymer in industrial applications specially in using for hospital bedding and fixtures. This

results is important due to less growing up on the surface of LDPE and for knowing that poly ethylene is one of most attractive medium to bacterial growth and adhesion beside it's ability of inducing genetic transformation in both bacteria (specially Escherichia coli) and yeast (specially Saccharomyces cerevisiae) without cell wall removal(18) .

This result could be explained due to a portion or all of lignin was not released from the film sample and diffuse into the agar layer yet it seems that of keep inside and near the surface of the film samples.

For *P.aeruginosa* and *K. spp.* The bacterial growth were dense all over the Petri dish with no adhesion around discs or on the surface of the film sample of the polymeric composite .After 48 hours of observing of the bacterial growth no big changes were recorded accept a slight adhesion with 3 mm in diameter of *S. aureus* on LDPE disc where this film sample was modified with 2% of lignin. *P. aeruginosa* has a dense growing with the beginning of colonies death . This result indicates some kind of bacterial or microbial corrosion to low density polyethylene due to the need of new sources of carbon (in this case polyethylene is rich) .

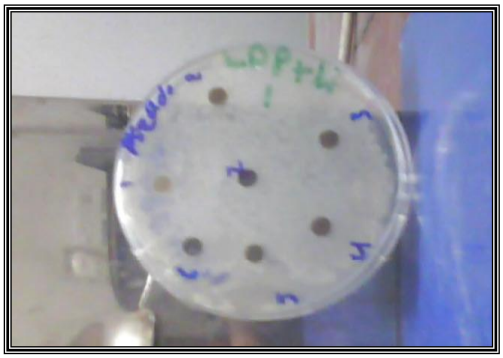
The obtained results of(lignin - low density polyethylene) composite may be explained by the act of packing up of polyethylene fibers The treatment of the pure polyethylene with Lignin forms a polyethylene/lignin mixture with tight surface , leading to a film with non dispersed agent (13). In such polymeric films bacterial growth depending on the physico-chemical properties of the microbial and biomaterial surfaces (lignin in this case which comes from plant sources) and as a result minimum loss of the active agent (lignin) is occurred and the higher melting temperature of this polymer with higher processing temperatures resulting in a much greater loss of lignin during processing (5). Also lignin is acting as a binding agent when combined with plastic according to it is ionic forces ; such forces is an extra factor to preventing attachment of testing bacteria on low density polyethylene discs this acting is similar to both gold and silver ions (14-15) .Furthermore, the researcher proved that the cell wall of bacteria is having negative electrical charge and the interaction between bacteria and other material depend on specific interactions, such as electrostatic and Lifshitz–Vander Waals forces, hydrophobic interactions and a variety of specific receptor–adhesin interactions (16)

and hence lignin is having many working groups (polarizing effect) that effecting on bacteria. Figure (2) shows the growth of tested bacteria. we can conclude that The use of cationic and anionic polymers can eliminate bacterial growth and adhesion on the surface of low density polyethylene – lignin composite due to the balance between two forces first the much working groups (cation and anion) we have in lignin where this force is acting

against bacteria ,second the tightness of LDPE modified by lignin where this tightness preventing these working group from diffusion in to the agar layer . more lignin concentration is required to achieve and improve antibacterial LDPE in order to improve the distribution of lignin with in the agar media .AS a final result ,there were no significant difference between pure LDPE and additive- Pure LDPE films in tested bacteria.

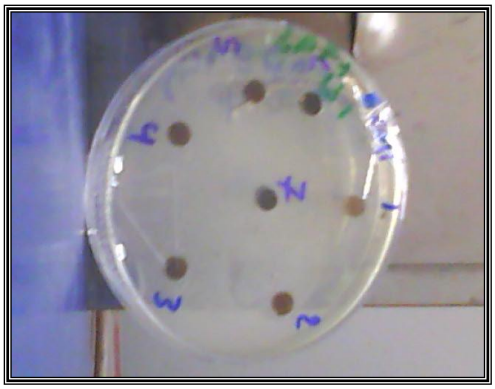
CONCLUSION

The results of the present study show that LDPE-based films containing natural lignin have no inhibitory effect for microbial growth in a nutrient agar containing a natural level of bacterial contamination .The impregnation of active components(lignin) into LDPE films would lower their diffusion and lead to higher concentration of the compounds on the surface of LDPE . It seems that the agar disc diffusion assay on packing films with typical test strains of microorganisms provides limited information on the effectiveness of the film thus the LDPE-lignin composite posses no inhibitory characteristics against tested bacteria .This lack of action stems probably from the fact that the concentration of lignin in the films was below the values required for demonstrating inhibitory effect against these organisms. Additional studies are required to investigate the loss of lignin in to the agar media.

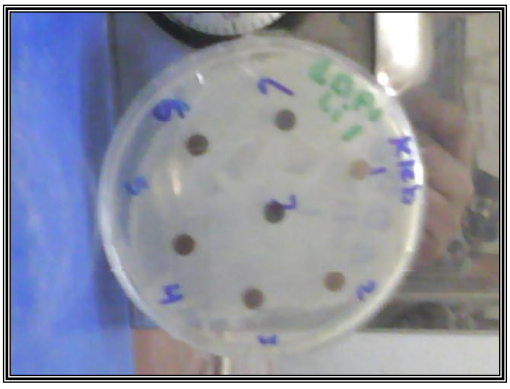


Pseudomonus aerugenosa

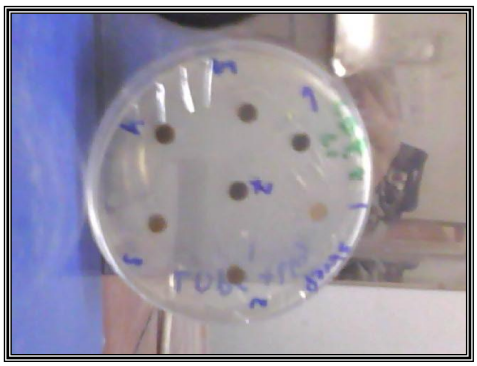
Streptococcus pyogens



Eeschrishia coli



Klebciala spp



Eeschrishia coli

figure (2) the growth of tested bacteria as a function of lignin concentration

دراسة النمو البكتيري على أقراص متراكبات بوليمر الاثيلين الواطئ الكثافة ـ اللكنين

نورس نوري جابر

فرع الإحياء المجهرية، كلية الطب البيطري، جامعة البصرة،البصرة،العراق.

الخلاصة

تم التحقق من مقاومه بوليمر الاثيلين الواطئ الكثافه والمطعم باللكنين النمو البكتيري باستخدام طريقه الانتشار بواسطة القرص الصلب حيث كانت نسبه المضاف الوزنيه من اللكنين الى هذا البوليمر مساوية إلى 1 % و2% و 3% و 4% و5% و10 % واخيرا 15 % من وزن بوليمر الاثيلين ب استخدام خمسه أنواع من الجراثيم وهي *staphylococcus aureus , streptococcus pyogens, pseudomonus aerugenosa, eschrishia coli and klebciala spp.*

وتم حقن هذه الاقراص ذات قطر 5 ملم في الإطباق الزجاجية ذات قطر 9 سم لمده تراوحت بين (1-2) يوم ومراقبه وتسجيل منطقه منع النمو البكتيري وبينت النتائج بعد مرور 24 ساعه ان النمو البكتيري لكل من *S. aureus* ، *S. pyogens and E. coli* مع عدم تسجيل أي نمو على سطح الاقراص البوليمريه في حين كان نمو كل من *P. aeruginosa* و *K. spp.* كثيفا في الاطباق . وبعد مرور 48 ساعة لوحظ وجود نمو خفيف على الأقراص البوليميريه وبقطر نمو 3 ملم مصحوبا بنمو كثيف لبكتريا *P. aeruginosa*

ووجد أيضا إن اللكنين المضاف ليس له تأثير واضح على البكتريا المستخدمة في هذا البحث وتم تفسير النتائج بالاعتماد على محدوديه طريقه الانتشار بواسطة القرص وان غياب منطقه منع النمو البكتيري كان مصحوبا بغياب النمو البكتيري على هذه سطوح أقراص البولي اثيلين الواطئ الكثافه ولابد من زياده تركيز اللكنين في هذه الاقراص للحصول على فعاليه ضد البكتريا المستخدمة

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