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Original Research Article

Effect of PVA, PVA/Biosurfactant on Some Pathogenic Bacteria in Glass and Plastic Plates

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A B S T R A C T

Staphylococcus aureus and *Pseudomonas aeurginosa* are some of the pathogenic bacteria able to form biofilm and then to produce surface associated infections. In this paper we studied the effect of PVA and PVA -Biosurfactant mixture on growth and biofilm formation of this bacteria in glass and plastic plates and study the influence of the molecular weight of PVA on their properties. Also, the optical absorbance spectra for pure PVA and Biosurfactant and there mixture were evaluated. Results showed that the best antibacterial activity against *Staphylococcus aureus* is with(PVA Mw 14000 blend biosurfactant for plastic plateat 100% Reduction of growth followed by the same mixture but in glass plate 98.7%), and against *Pseudomonos aeruginosa* is with (PVA Mw 160000,14000 blend biosurfactant for plastic plate 98%,98% respectively). The best anti-adhesive effect against *Staphylococcus aureus* are (PVA with Mw 160000 pure and mix with biosufuctant for both plastic and glass plates with inhibition ratio (40.19%,46.68%)for PVA in plastic and glass respectively and (73.53%,47.12%) for PVA \ Biosurfactant in plastic and glass respectively , and against *Pseudomonos aeruginosa* are pure PVA Mw 160000 in plastic 79.75% and PVA Mw 14000 \Biosurfactant in glass 23.34%. The UV-Visible absorbance spectra showed high level interference between PVA and Biosurfactant molecules , and this factor work to improve the antibacterial activity of PVA and give advanced results . To the best of our study this is the first report on antibacterial and antiadhesive effect of PVA and PVA- Biosufactant mixture against pathogenic bacteria in glass and plastic plates and study the influence of the molecular weight of PVA on their property.

Keywords

Biofilm; inhibition; *Staphylococcus aureus ; Pseudomonas aeurginosa* ; PVA ; biosufactant .

Introduction

Polymers may either be naturally occurring or purely synthetic. Enzymes, nucleic acids, and proteins are polymers of biological origin. Their structures are normally very complex, and natural rubber. There are a large number of synthetic (man-made)

polymers consisting of various families: fibers, elastomers, plastics, adhesives, etc. Each family itself has subgroups (Ebewele ,2000). Polymer have a great potential in many important applications because of their unique properties, such as low density, ability to form intricate shapes and low manufacturing cost (Abdullah,2011) .

Polyvinyl alcohol (PVA) isemulsifying and adhesive properties. It is also resistant to oil, grease and solvents. It is odorless , nontoxic, fully degradable and dissolves quickly (Eliassaf ,1972). PVAis synthetic polymer, innocuous, non-carcinogenic and have good biocompatible properties. Because of its excellent film forming and highly hydrophilic water-soluble with outstanding chemical stability,it is useful in many applications such as controlled drug delivery systems, recycling of polymers and packaging (Tripathi *et al*.,2009).

Biosurfactantsare microbial produced. These molecules have attracted considerable scientific attention due to lower toxicity, higher biodegradability, activity at extremes of temperature (Desai and Banat, 1997).The ability to reduce surface tension is a major characteristic of surfactants. Surfactants are key ingredients used in detergents, shampoos, toothpaste, oil additives, and a number of other consumer and industrial products (Anandaraj andThivakaran,2010). Biofilm is defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to an inert or living surface." Biofilm-producing organisms are far more resistant to antimicrobial agents than organisms which do not. Biofilms have great importance for public health because of their role in certain infectious diseases and a variety of device related infections (Gurung *et al*,2013).

Biofilms may form on a wide variety of surfaces, including natural aquatic systemsliving tissues, indwelling medical devices and industrial/potable water systempiping (Percival *et al.*,2011) .

The aim of this study is to investigate the effect of PVA and PVA-Biosufactant mixture on growth and biofilms formation of *Staphylococcus aureus* and *Pseudomonas aeurginosa* bacteria in glass and plastic plates and study the influence of the molecular weight of PVA on their property.

Materials and Methods

PVA –Biosurfactant films

Biosurfactant produced by locally *Lactobacillus rhamnosus* isolate obtained from al-Qaralucy(Department of Biology \ College of Science \ Al- Mustansiriya University \ Baghdad\ Iraq). PVA[-CH_2CHOH-]$_n$ Mw (160000,14000) from (HIMEDIA CO. India and DBH CHMICAL LTD POOLE ENGLAND) respectively.

PVA solution was prepared by blending 0.5g of PVA with 10ml distilled water , stirred for 3to 4 hours to ensure for fully dissolving.

Forming the (PVA –Biosurfactant) films: PVA with biosurfactant was mixed at ratio (1:1) (vol:vol) before casting the mix in platesand let them dry for 24 hours .

Antibacterial effect of PVA, PVA\ Biosurfactant films against pathogenic bacteria

Antibacterial effect of PVA films with a molecular weight (14000 and 160000) doped with biosurfactant were determined against pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aeurginosa* (obtained from Department of Biology/college of science / Al-Mustansiriya

University /Baghdad /Iraq). PVA and PVA\Biosurfactant were coated on glass and plastic Petriplates, then dried for (24 hours) . After drying the bacterial suspensions (10^8 cell/ml) are poured on to the film of glass and plastic plates and allowed to settle on the top of the film, the control plates contained bacterial suspensions only without PVA and PVA\Biosurfactant films. All coated plates and control were incubated at 37^oc for 24 h. After the incubation 1 ml of each dilution was taken and spreaded on nutrient agar (Hi-Media) and then incubated at 37^oc for (24 – 48) h. The colonies were counted and inhibition effect was evaluated and calculated percent reduction of bacterial growth using the followingequation described as(Gosh *et al.*, 2010) :

R(%)=[(A-B)/A] X 100

R = the reduction rate, A = the number of bacterial colonies from control plates and B= the number of bacterial colonies from plates coated with PVAor PVA \ Biosurfactant films

Anti-adhesive effect of PVA, PVA\ Biosurfactant films against pathogenic bacteria

The anti-adhesive effect of PVA and PVA\Biosurfactant films with a molecular weight of PVA (14000 and 160000) against pathogenic bacteria (the same bacteria that were used in the antibacterial effect) were determined by precoating experiment that explained in the antibacterial effect. After the incubation for (24)h of coated and control plates, unattached bacterial cells were removed by washing the plates three times with water, then drying at room temperature for 15 min. After drying crystal violet (1%) was added to the plates for 20 min. the stained attached bacterial cells were rinsed three times with distilled water, allowed to dry at room temperature for 15 min and extracted twice with 95% ethanol (Ali,2012 with modification) and the absorbance was measured at 590 nm using spectrophotometer. The inhibition of adhesion percentages were calculated as equation described by (Gudina *et al* .,2010).

% inhibition of adhesion = $[1- (A/A_o)]x 100$
Arepresents the absorbance of the plates coated with PVA or PVA\Biosurfactant
A_o the absorbance of the control plate

This method of anti-adhesion assay estimates the percentage bacterial adhesion reduction in relation to the control plates, which were set at 0% to indicate the absence of PVA, PVA\Biosurfactant and therefore of its anti-adhesion properties. In contrast, negative percentage results indicate the percentage increase in microbial adhesion at the presence of PVA, PVA\Biosurfactant in relation to the control.

Results and Discussion

The good biological characteristics of films (PVA \ Biosurfactnat) attributed to good effect of PVA and boisurfactant separately . The objective of this research was to study and evaluate the antibacterial activity of PVA , PVA \ Biosurfactnat films on pathogenic bacteria included *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The process of implant bacteria in Plates contain films of polymer and polymer blend with biosurfactant for a different molecular weights of PVA 160000 g/mol and 14000 g/mol give good results to eliminate bacteria (gram positive and gram negative) in case of polymer alone ormix, also PVA / PVA-biosurfactant mixture give a good result for inhibition of biofilm formation for those pathogenic bacteria which have the ability to form biofilms.

The results showed that the best antibacterial activity against *Staphylococcus aureus* is with PVA Mw 14000 blend biosurfactant for plastic plate 100% reduction of growth followed by 98.7% for glass plate (Table-1-) and against *Pseudomonas aeruginosa* is with PVA Mw [160000,14000] blend biosurfactant for plastic plate 98%(Table-2-) While the best anti-adhesive effect against *Staphylococcus aureus* are (PVA with Mw 160000 pure and mix with biosurfuctant for both plastic and glass plateswith inhibition ratio(40.19%,46.68%)for PVA plastic and glass respectively and (73.53%,47.12%) for PVA \ Biosurfactant plastic and glass respectively(Table -3-), and against *Pseudomonas aeruginosa* are pure PVA Mw 160000 plastic 79.75%and PVA Mw 14000 \Biosurfactant glass 23.34%, (Table.4).

UV-Visible absorption spectra for both PVA with two molecular weights, Biosurfactant , and mixture of these two materials are shown in Figs.(1, and 2).

The maximum wavelength of absorption spectrum of PVA polymer with two molecular weight is at 280nm, and this is matched with results obtained by Mahdy et al., (2013) , Abdullah and Hussen (2011) and Rabee(2011) they also study the optical properties of PVA showed a peak intensity of absorbance are (0.9 , 0.01,0.4) appear at (270, 280,280 nm) respectively .

Whereas the intensity of absorption spectrum of PVA increased with decreasing molecular weight of polymer; 0.204for 160000 and increased to be 0.519 for lower Mw= 14000.

The absorption spectrum of biological material Biosurfactant has wider peak with maximum wavelength at 515nm with intensity 3.131, as shown in figs. (1 and 2).

While the absorption spectrum for mixture solution (PVA and Biosurfactant) has no peaks but only curve for decreasing absorbance range from 490 to 665nm for higher molecular weight and shifted to lower wavelength 465 to 665nm with decreasing Mw.

These results indicated that there is high overlap between PVA and Biosurfactant molecules which improve antibacterial activity than each one separately. To explain what happen between bacteria and polymer; the antimicrobial activity of the compounds is related to cell wall structure of the bacteria. Because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan as mentioned by Hosny and Khalaf-Alaa (2013). From our results it may be concluded that the PVA,Biosurfactant and their mixture effect on biofilm they could penetrate the surface of bacterial Pyramidal wall so that they can disassemble and prevent bacteria to form biofilm consequently.

Many authors study the effect of biosurfactant and reach to there is a good activity of biosurfactant against bacteria Aziz *et al.* (2014) , also al-Qaralucy(2014) showed that the crude and partial purified biosurfactant isolated from *Lactobacillus rhamnosus* had antibacterial, antibiofilm and antiadhesive properties against some bacteria *(Klebsiella pneumoniae, Burkholderia cepacia, Escherichia coli* and *Staphylococcus aureus*), but this less percentage compared with that obtained in our studyafter blending biosurfactant with PVA. Rodrigues *et al*. (2006) showed that the main goal of biosurfactant is to modify the physicochemical properties of the surface in order to reduce the force of attraction between microorganisms and the

surface of the biomaterial. Gudina *et al*. (2010) and Brozozowski *et al*.(2011) showed that the highest antiadhesive percentages were obtained for *Staphylococcus aureus, Staphylococcus epidermidis Streptococcus agalactiae* for a biosurfactant isolated from *Lactobacillus paracasei*, and a low activity was observed for *Pseudomonas aeruginosa* and *Escherichia coli*. Salman *et al*. (2013) observed that the crude biosurfactant isolated from *Streptococcus thermophilus* showed inhibitory effect against *Klebsiella* spp. and *Pseudomonas aeruginosa*. The antimicrobial activity of the crude biosurfactant isolated from *S. thermophilus* and *Lactococcus lactis* observed against *S. aureus* and *S. epidermidis* was which completely inhibited the growth of those bacteria with concentrations 100 mg/ ml (Rodrigues *et al.*, 2004). Another studies, biosurfactant isolated from *B.subtilis, B.licheniformis* and *Pseudomonas aeruginosa* showed inhibition activity against gram positive and gram negative bacteria (Gomaa 2012,Ghribi*et al*, 2012, Lotfabad*et al*., 2013).Dhanalakshmi *et al*. (2011) and Yang *et al*.(2010) showed the antimicrobial activity of nanocomposit of hydroxyapatite \ PVA. Hosny and Khalaf-Alaa (2013) they screened the antimicrobial activities of PVA ligand and their metal chelates ions Cu(II), Ni(II), Co(II), and Zn(II) using the disc diffusion method against the selected gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*),The antibacterial activity results indicated that tested complexes were more active against the selected types of bacteria than the free PVA ligand. Tripathi *et al.* (2009) showed that the use of an antimicrobial coating consisting of chitosan–PVA is a viable alternative in shelf-life extension of minimally processed tomato .Chitosan-PVA antimicrobial film may be a promising material as a packaging film. Ryparova *et al.*(2012) observed the antibacterial activity of PVA against *Eschelichia coli* and the PVA membrane reduces bacteria reproduction.

Abdelrazek *et al* (2012) study the optical properties of PVA , they showed a peak absorbance appear at 210 nm shifts gradually towards the higher wavelength side with increasing concentration of dopant.

Table.1 Antibacterial effect of PVA, PVA\Biosurfactant against *S.aureus* in glass and plastic plates

TRETMENT	Mw of PVA	Type of plates	Reduction percentage(%)
PVA	160000	plastic	84.68%
PVA	160000	Glass	76.37%
PVA	14000	plastic	26.60%
PVA	14000	Glass	87.50%
PVA + BIO	160000	plastic	82.88%
PVA + BIO	160000	Glass	46.45%
PVA + BIO	14000	plastic	100.00%
PVA + BIO	14000	Glass	98.70%

Table.2 Antibacterial effect of PVA, PVA\Biosurfactant against *P. aeruginosa* in glass and plastic plates

TRETMENT	Mw of PVA	Type of plates	Reduction percentage(%)
PVA	160000	plastic	86.20%
PVA	160000	Glass	58.57%
PVA	14000	plastic	42.85%
PVA	14000	Glass	57.10%
PVA + BIO	160000	plastic	98.00%
PVA + BIO	160000	Glass	97.70%
PVA + BIO	14000	plastic	98.00%
PVA + BIO	14000	Glass	87.14%

Table.3 Anti-adhesive effect of PVA, PVA\Biosurfactant against *S.aureus*

TRETMENT	Mw of PVA	Type of plates	Inhibition of adhesive(%)
PVA	160000	plastic	40.19%
PVA	160000	Glass	46.68%
PVA	14000	plastic	-243%
PVA	14000	Glass	-222%
PVA + BIO	160000	plastic	73.53%
PVA + BIO	160000	Glass	47.12%
PVA + BIO	14000	plastic	-195%
PVA + BIO	14000	Glass	-73.30%

Table.4 Anti-adhesive effect of PVA, PVA\Biosurfactant against *P. aeruginosa*

TRETMENT	Mw of PVA	Type of plates	Inhibition of adhesive(%)
PVA	160000	plastic	79.75%
PVA	160000	Glass	-11.00%
PVA	14000	plastic	-260.00%
PVA	14000	Glass	-2.00%
PVA + BIO	160000	plastic	-14.60%
PVA + BIO	160000	Glass	-389.90%
PVA + BIO	14000	plastic	-300.00%
PVA + BIO	14000	Glass	23.34%

Fig.1 UV/VIS spectra of PVA(Mw 160,000),Biosurfactant and mixture of both .

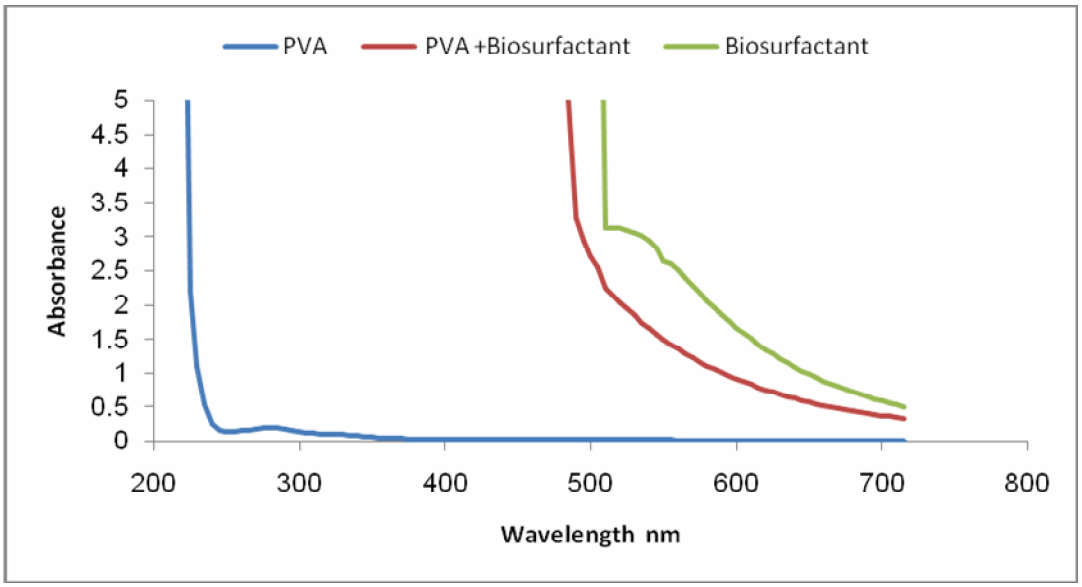
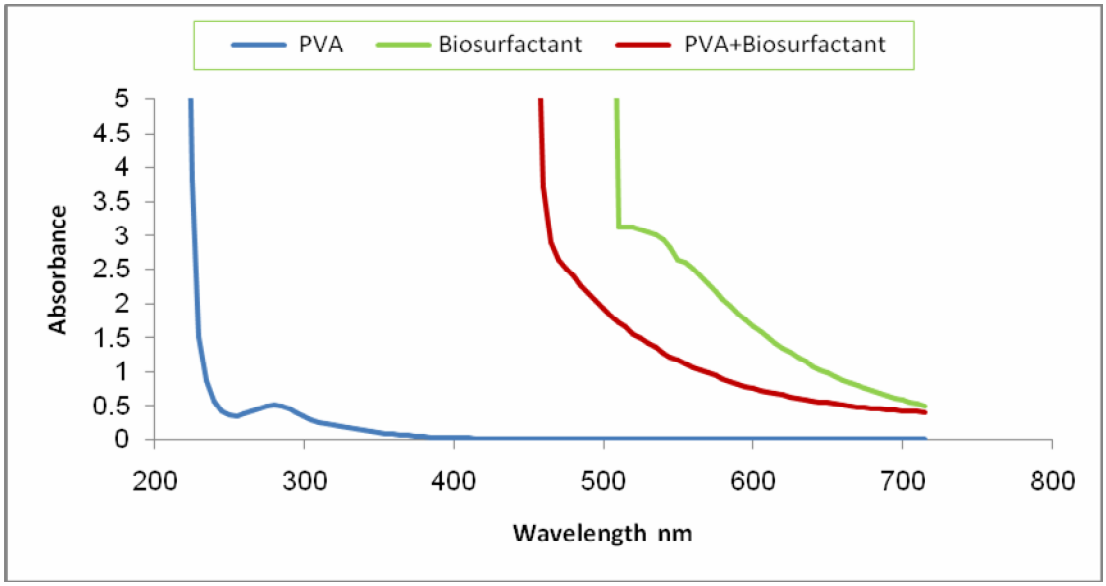


Fig.2 UV/VIS spectra of PVA(Mw 14,000), Biosurfactant and mixture of both



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