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Effect of cell surface hydrophobicity in microbial biofilm formation

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ABSTRACT

Cell surface hydrophobicity may be regarded as most relevant parameter for the assay of microbes for their biodegradation capability and suggested for the screening of such microbial strains from the mixed population. The cells having more hydrophobic nature usually show maximum capability of biofilm formation and biodegradation. Large numbers of bacteria attached to hydrophobic plastics with little or no surface charge, polyethylene, polystyrene, moderate numbers attached to hydrophilic metals with a positive, or neutral surface charge; and very few attached to hydrophilic, negatively charged substrata. The study that determination of cell surface hydrophobicity is helpful to decide the strain having higher capability to biodegrade LDPE if it maintains higher hydrophobicity.

Keywords: LDPE biodegradation, Hydrophobicity, Biofilm, Surface active compounds

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INTRODUCTION

Solid surfaces that are in contact with water in natural and man-made environments are rapidly colonized by bacteria. This pervasive colonization of surfaces by bacteria and the formation of bacterial biofilm or biofouling communities have important implications for ecological function, industrial processes, and human health [1]. Costerton et al. [2] define a biofilm as “a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.” The molecular nature of the bacterial cell surface is crucial in the interaction between the micro-organisms and the host [3]. Generally microbes have already been observed to form the biofilm under certain circumstances like nutrition cues, inhibitory gents like antibiotics or toxins [4, 5] i.e. under threat conditions thus may be termed as outcome of phenomenon to form oriented structure for protection and survival. This ability to form the protective structure provides several advantages like increased access to nutrient, protection against toxins and antibiotics, maintenance of extracellular activities and shelter from predation [6].
In the present study, the cell-surface hydrophobicity of two *Pseudomonas* sp. were tested for the biofilm formation.

MATERIALS AND METHODS

Microbial strains source

The microbes were isolated from LDPE films collected from the solid waste dump region.

Identification of test organisms

Primarily isolated organisms were subjected to biochemical tests and identified through morphological characters.

LDPE powder

Low density polyethylene films were obtained from B.N. Polymers, India and dried overnight in hot air oven at 60^0C to obtain dry powder, stored at room temperature for further use.

Culture Preparation

Cultures were grown using the synthetic medium [NH_4NO_3 1.0, K_2HPO_4 0.7, KH_2PO_4 0.7, $MgSO_4.7H_2O$ 0.7, NaCl 0.005, $ZnSO_4.H_2O$ 0.002, $MnSO_4.4H_2O$ 0.001, $FeSO_4.H_2O$ 0.002] [g/l] supplemented with 0.3% LPDE powder for the study of cell surface hydrophobicity and other parameters.

Cell surface hydrophobicity evaluation

5ml of 24hr old culture was taken, centrifuged, pallets was re-suspended in Phosphate-magnesium buffer, centrifuged supernatant pooled into one, OD was taken at 400nm using UV-VISIBLE spectrophotometer-8500 II called Initial Bacterial Suspension. Again 5ml of culture was taken and mixed 0.2ml of Hexadecane. Mixed to get two phases, OD was taken at 400 nm for aqueous phase called as Final concentration in aqueous phase [7].

Biofilm quantification

Three LDPE films were taken and disinfected with 70% ethanol for 30 min, washed with distilled water for 10 min, taken into 100ml synthetic media inoculated with 24 hr old culture, kept in magnetic stirrer. After every 2 days, 1 LDPE film was taken washed in 10% ethanol by vigorous shaking. OD was taken at 540nm using UV-VISIBLE spectrophotometer-8500 II, 95% ethanol served as blank [8].

RESULTS AND DISCUSSION

Screening and identification of LDPE degrading bacteria

Both the microbes were identified as *Pseudomonas* sp. as per the morphological characters.

Bacterial Hydrophobicity

It was found that *Pseudomonas*-2 strain was more hydrophobic in nature i.e. 75%, showed highest capability to form biofilm [Fig. 1] as observed through protein concentration level i.e. 98µg/ml and facilitated efficient biodegradation as compared to strain *Pseudomonas* -1.

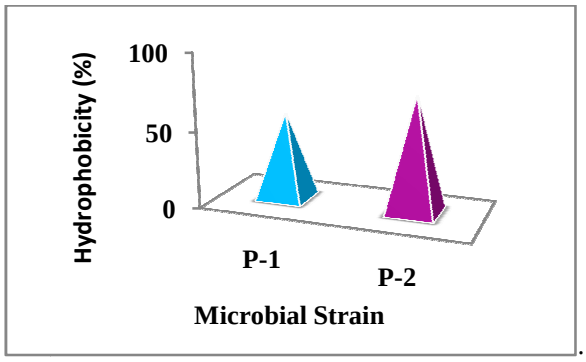

Fig. 1: Hydrophobicity percentage

The bacterial isolate *Pseudomonas*-2 have significant colonization than *Pseudomonas*-1, resulting formation of dense biofilm. As large numbers of bacterial cell of *Pseudomonas* -2 were attached on the LDPE, the level of extracted protein concentration from the film was high (98µg/ml) (Figure 2) and facilitated efficient biodegradation of as compared to strain BSM-1 prototype.

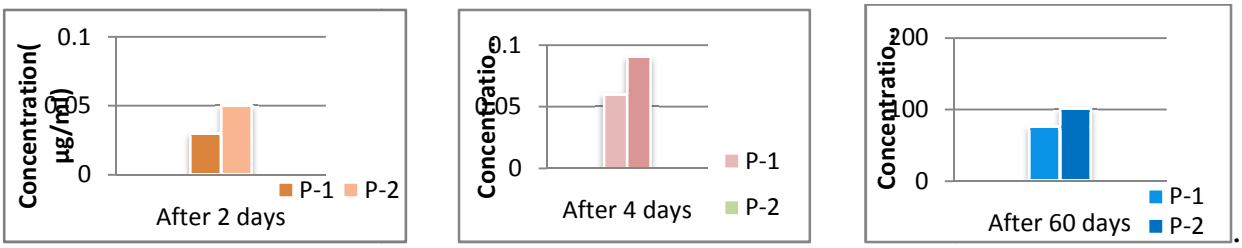

Fig. 2: Determination of protein concentration from colonized film

The evidences from published research, taken together with suggested approach could give a satisfactory explanation for this behavior of cells. The hindrance offered by hydrophobic film is nothing but the formation of the

interfaces of water and hydrophobic surface due to repulsion of the duo facilitated due to opposite nature of surface charge. In this respect surface active compounds (SACs) have found to play a vital role to help the microbes to interact through interfaces [9] by forming conditional film which is mainly composed of lipids, proteins, complex polysaccharides and humic substances [10].

CONCLUSION

The nearly universal colonization of surfaces in marine waters by bacteria and the formation of biofilms and biofouling communities have important implications for ecological function and industrial processes. These surface-associated microorganisms contribute substantially to degrade the xenobiotic compounds present on the attached surfaces and influence the biodegradation of toxic polymers.

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