Super-hydrophobic Paper Influence on Bacterial Attachment Suggests Potential Application as Packaging Material

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Abstract:

The surface topography of cellulose-based paper was altered by plasma etching and fluorocarbon polymerization that led to surface superhydrophobicity (water contact angle (CA) >160). This surface modified paper is biodegradable, renewable, and inexpensive and has been shown to influence bacterial attachment. Two types of modified papers have been studied for their bacterial attachment properties, one with low hysteresis (LH) and the other with high hysteresis (HH); both were compared with results on untreated paper. Reduction of bacterial count after dropping suspensions of *Salmonella typhimurium* on HH and LH papers has been observed. The average values of the six replicates of the colony forming units for the untreated, HH and LH have been found to be $6.4*10^7$, $1.4*10^7$ and $0.5*10^7$ respectively. Elemental analysis of the papers was done using Laser induced break down spectroscopy (LIBS). A single laser pulse from a Nd:YAG laser operating at 532 nm was used for the analysis. The atomic emission was approximately 2-3 times higher from the LH paper as compared to HH paper. LIBS offers a relatively quick method of differentiation between the types of papers. The relative intensities for both atomic and molecular emission can be used to characterize the treated and untreated papers as a substantial difference in the intensities has been observed. The modified paper could have potential applications in a vast array of products including microwavable food packages and self-cleaning cartons.

Key words:

superhydrophobicity, bacterial attachment, laser-induced break down spectroscopy

1. Introduction:

Packaged food is often contaminated by pathogenic microorganisms during the manufacturing and packaging processes. Thus, food packaging has considerable importance to public health. There is an increasing concern over the bacterial contamination arising from bacterial biofilms which develop on the material used during food manufacturing process [1]. Bacteria often attach to the organic polymers exuded by the cells; these bacteria can be difficult to remove, require time consuming and expensiv efforts to remove. The formation of bacterial biofilms takes place in three stages; a reversible adsorption, adhesion of bacteria to the surface and lastly colony formation [2]. In order to prevent or retard the process of biofilm formation the use of materials to which bacteria cannot initially attach, would be of commercial interest [3]. Cellulose-based paper is chosen as the candidate for making a superhydrophobic surface because it is biodegradable, renewable and abundantly present in nature. This will offer a promising alternative to conventional superhydrophobic substrates that could have applications in a vast array of products, including fast food, microwavable food packages, and self-cleaning cartons [4].

In this paper we demonstrated that superhydrophobic paper, prepared by selective etching of cellulose by an oxygen plasma and followed by plasma-enhanced chemical vapor deposition (PECVD) of a fluorocarbon film (~100 nm) influenced bacterial attachment.

2. Materials and Methods:

Salmonella enterica serovar Typhimurium LT2 was purchased from ATCC (13311) and grown in brain heart infusion broth media (BHI, purchased from Sigma-Aldrich) overnight. LH, HH and untreated papers placed in separate petri dishes were spotted with 1 ml of 10^7 cfu/mL of *Salmonella* grown over night (Figure 1).

After incubation at 37° C for 20 minutes, the papers were dipped in 2 ml of 1 x PBS to get all the bacteria into solution. As received bacteria were plated in triplicate on plate count agar plates using automated spiral plater (Spiral Biotech Advanced Instruments Inc.) and colony forming units (cfu) were counted using Q count (Advanced Instruments Inc.).

Figure1. Hydrophobicity of the HH and LH papers (first two sets respectively) compared with the untreated paper (last set).

An Nd:YAG operating at 532 nm was used in the LIBS analysis. Tape was used to ensure that the paper laid flat on a surface during the LIBS analysis. A single laser shot was used to ensure that the emission obtained was only from the paper substrate. The emission from the laser-produced plasma collected perpendicular from the laser beam. The light was recorded approximately 500 ns after plasma ignition and integrated over 5 µs. After one measure was made on the paper, the paper was moved to fresh spot and another measurement it taken. In this fashion, 9 LIBS spectra were collected for both the LH and HH paper.

3. Results and Discussion:

Reduction of bacterial count after dropping suspensions of *Salmonella typhimurium* on HH and LH papers has been observed. The average values of the six replicates of the cfu's for the untreated, HH and LH have been found to be $6.4*10^7$, $1.4*10^7$ and $0.5*10,^7$ respectively (Table 1)

Replicates	Number of Colonies	Cfu/mL	Percent Reduction
Replicate 1			
Untreated	25	$2.5*10^7$	88.263
HH	4	$0.4*10^7$	98.122
LH	3	$0.3*10^7$	98.592
Replicate 2			
Untreated	85	$8.5*10^7$	60.094
HH	14	$1.4*10^7$	93.427
LH	18	$1.8*10^7$	91.549
Replicate 3			
Untreated	34	$3.4*10^7$	84.038
HH	17	$1.7*10^7$	92.019
LH	5	$0.5*10^7$	97.653

Table1. Reduction in bacterial count after dropping suspension of *Salmonella* bacteria on treated and untreated papers.

Laser induced breakdown spectroscopy is a useful tool to measure surface changes. The technique is applicable to a wide range of surfaces and materials and provides good information about elemental composition. In this study, LIBS was used to perform the elemental analysis of the papers. LIBS has been shown to provide good discrimination of papers made at various plants using Na, Mg, Al, Ca, and Sr [5].

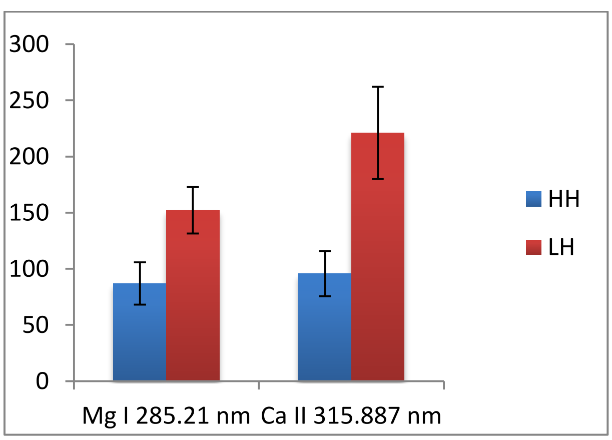

Figure 2. Comparison of elemental analysis done for the HH and LH papers using LIBS.

Figure 2 compares the elemental analysis done for the HH and LH papers using LIBS.

As can be seen in the figure, there is a significant difference in the elemental emission intensities of two types of superhydrophobic papers. Although only Mg and Ca are shown, elements such as Na gave similar results.

Thus, the LIBS assay offers a relative quick and simple way of differentiating between the types of papers used in this study. In future, the LIBS assay can also be used to study the differences in bacterial attachment seen with the different types of papers that have been developed.

Overall, the results indicate that these superhydrophobic papers should find application in packaging, printing, de-inking and other chemical and biochemical industries.

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