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Formation/Removal of Biofilms on/from Coupons of Selected Food-Grade Elastomeric Polymers vs. Plexiglass Used for the Fruit-Catching Plates of OTR Blueberry Machine Harvesters

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Abstract: To reduce the bruising potential of machine-harvested fresh blueberries, manufacturers of over-the-row (OTR) machine harvesters are replacing the hard, plexiglass fruit-catching plates with soft, elastomeric polymers. This study assessed whether selected soft, food-grade elastomeric polymers, with the potential to be used in OTR harvesters, have a greater likelihood to encourage more microbial buildups, making cleaning/sanitation a greater challenge. Coupons of plexiglass, silicone, neoprene, and ethylene propylene diene monomer (EPDM) were exposed to fecal coliforms from various sources for biofilm development. The coupons with developed biofilms were treated with sodium hypochlorite, peracetic acid, isopropyl alcohol-based quaternary ammonium compounds (Alpet D2), or commercial dish soap. Biofilms and their residuals after the sanitizer treatments were quantified. The fecal coliforms isolated from the surface of OTR harvesters developed significantly ($p \leq 0.05$) more biofilms than those from other sources. EPDM coupons had significantly more, while neoprene and silicone coupons had insignificantly different ($p > 0.05$) amounts of biofilms from plexiglass coupons. After sanitizer treatments, EPDM coupons had significantly more, while neoprene and certain silicon coupons had significantly fewer residues than plexiglass coupons. Study suggests that compared to plexiglass, neoprene and silicon did not support more microbial buildups or retain more biofilms after sanitizing treatments.

Keywords: food-grade elastomeric polymers; plexiglass; fruit-catching plates; OTR harvester; biofilm formation

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1. Introduction

Blueberries (*Vaccinium* spp.) destined for the fresh market are primarily harvested by hand [1]. Studies have shown that hand-harvested blueberries are of better quality with a firmer texture and longer postharvest shelf lives [2]. However, due to an increase in production scale, lack of worker availability, and increase in labor costs, more growers are transitioning to using over-the-row (OTR) machine harvesters to harvest blueberries for the fresh market [3].

During mechanical harvest using OTR harvesters, plastic beating bars or rods mounted on rotary shaking drums inside the machine tunnel cause blueberries to separate from the bushes [4]. Separated berries fall onto either side of the fruit-catching plates before being transported by horizontal conveyors to small buckets at the rear of the machine. Buckets with harvested berries are subsequently lifted to a platform by a vertical bucket conveying system. Plant debris and leaves are removed by air blowers before lifted berries fall into harvest lugs.

The OTR machine harvesters have been found to produce fruits unsuitable for the fresh market, particularly if the fruit needs to remain in postharvest storage for some time [2]. The hard surfaces of machine harvesters (e.g., the plexiglass fruit-catching plates) created significant damage to harvested berries with increased bruising and reduced firmness compared to hand-harvested fruit [4,5]. Consequently, blueberries harvested by conventional mechanical harvesters have been found to have relatively shorter shelf lives [6], which may adversely affect the revenue that blueberry growers can make from fresh market blueberries.

OTR machine harvester manufacturers and collaborating researchers have experimented with substituting soft, food-grade elastomeric polymers for the hard, plexiglass fruit-catching plates of the machine [7,8]. Yu et al. [5] reported that the modification significantly reduced the physical impacts on harvested blueberries. However, some of the substituting soft, food-grade elastic polymers have relatively high surface hydrophobicity, which may attract bacterial cells for colonization. The colonized bacteria, be they spoilage-causing or pathogenic, could dislodge from the machine surface when conditions permit, causing the contamination of fresh market blueberries. The goal of this study was to determine the feasibility, from a microbiological standpoint, of using selected soft, food-grade elastomeric polymers to modify the hard, plexiglass fruit-catching plates of OTR blueberry machine harvesters. This goal was accomplished by the examination of biofilm mass accumulated on surface coupons of plexiglass vs. selected food-grade elastomeric polymers and the efficacy of selected chemical treatments in removing accumulated biofilms under laboratory conditions.

2. Materials and Methods

2.1. Bacterial Strains Used in the Study

Biochemically confirmed fecal coliforms (n = 9) previously isolated from fruit-packing environments were used in the study (Table 1). The isolates were retrieved from −80 °C freezers and resuscitated on tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD, USA) plates at 37 °C overnight. The resultant cultures were sub-cultured twice under the same growth conditions.

Table 1. Bacterial strains used in the study.

Inoculum	Isolate ID	Year of Isolation	Source of Isolation
I	1212	2015	Fresh fruits
II	1238/1243/2470	2017	Hand gloves
III	2901/2902	2017	Packing lines
IV	2561	2015	Packing lines
V	177/178	2015	Harvest machines

The identities of the fecal coliform isolates were confirmed by 16S rDNA sequencing using universal primers 27F and 1492R and a method described by Gazula et al. [9]. Amplified PCR products were submitted to Eurofins Genomics, a Eurofins MWG Operon Company (Louisville, KY, USA), for purification and sequencing. The acquired 16S rDNA sequences were compared with those in the NCBI database. Potential virulence and putative adhesion genes in the fecal coliform isolates were screened using the PCR conditions and oligonucleotide primers described by Toma et al. [10] and Low et al. [11], respectively. The sequences of the primers for the screened genes are shown in Table 2.

Table 2. Oligonucleotide primers used for the screening of virulence, and putative adhesin, genes [10,11].

Primer	Target	Primer Sequence	Amplicon (bp)
SK1 SK2	*eae*	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	881
VT com-u VT com-d	*stx*	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518
AL 65 AL 125	*est*	TTAATAGCACCCGGTACAAGCAGG CCTGACTCTTCAAAAGAGAAAATTAC	147
LTL LTR	*elt*	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT	322
Ipa III ipa IV	*ipaH*	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	619
aggRks1 aggRkas2	*aggR*	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254
Loc 1	*stc*A	5′-CGACAACGTTGATGTTTAGC 3′-GCCTTTTGTAACAGGATTGC	300–500
Loc 2	*yad*N	5′-GGTATGCATAGCGTTACC 3′-CTGCTGGCAAATCTTATGC	300–500
Loc 3	*sfm*A	5′-GCGGTACAATTCACTTTGAAGG 3′-CATTTGCTTGCCCTGCTGATGC	300–500
Loc 4	*ybg*D	5′-GCCATATCTCTACTATTCGC 3′-GTTATCCATCTGTTCCATCC	300–500
Loc 5	*ycb*Q	5′-CTGTGGTATGTGCAACGTCC 3′-CCCCGTAGCGATATAATCAAC	300–500
Loc 6	*sfa*A	5′-CCTACAGTCACTTTTCAGGG 3′-GATTAATTAGAGGTAGCTCAGG	300–500
Loc 7	*csg*A	5′-CTTCATTTAATCAGGCAGCC 3′-GAGTACCATACTGTGTAATATTTGC	300–500
Loc 8	*fim*A	5′-GGTGATGAATCAGTAACGACC 3′-GTGCCATCAATCAAGTCGG	300–500
Loc 9	*yeh*D	5′-CACCATGTACATTTGTCGC 3′-CAGTACGTCACTGCTATCTCC	300–500
Loc 10	*stf*G	5′-GCTGCAACAATGGTAATGGG 3′-GTAATCTGGAAGGTCGTGTTGGC	300–500
Loc 11	*yra*H	5′-CTTTTCGCAGGTAATGCCG 3′-GATTTCGGATGCTTCAACG	300–500
Loc 12	*lpf*A	5′-GTGGTATCGCAATCTTCC 3′-GGTAAAGTAGAGAACCG	300–500
Loc 13	*lpf*A	5′-GATTGTAGGAGCATTAGCG 3′-CTATCGATCTGACTCAATGCC	300–500
Loc 14	*fim*A	5′-GTCGTTGCTGCCAATGTTTGC 3′-GAAATGTAGCGAAGTAGAGCC	300–500

2.2. Surface Coupons Used in the Study

Plexiglass (polymethyl methacrylate), the traditional material used to manufacture the fruit-catching plates of OTR machine harvesters (Oxbo International Corporation, Lynden, WA, USA), along with four food-grade elastomeric polymers with a smooth texture, were used in the study (McMaster-Carr, Elmhurst, IL, USA). These include neoprene (polychloroprene; item no. 8616K64), silicone (polysiloxane; white colored with item no. 86045K79 and red colored with item no. 1460N22), and EPDM (ethylene propylene diene monomer; item no. 8143K141). The elastomeric polymeric sheets were purchased in October 2019 and were cut into 2 × 5 cm coupons upon receipt. The coupons were first cleaned with an alkaline detergent (Fisher Scientific, Pittsburgh, PA, USA) and washed with tap water, followed by soaking in a 70% ethyl alcohol solution, rinsing with distilled water, and drying at ambient temperature. The plexiglass coupons were subsequently decontaminated in a 5% hypochlorite solution for 1 h, rinsed three times with sterile deionized water, and dried in a level II biosafety hood. The neoprene, silicone, and EPDM coupons were, nevertheless,

autoclaved at 121 °C for 15 min and dried at the same temperature for 15 min. The same batch of polymeric materials was used throughout the experiments described in the study.

2.3. Biofilm Formation on Surface Coupons

Single (culture I and IV) or mixed (culture mix II, III, and V in 1:1 or 1:1:1 ratio in cell population) fecal coliform cultures were permitted to develop biofilms on each of the surface coupons in Luria-Bertani no salt broth at 25 °C for 7 days. An un-inoculated broth was included in the study as a control. Biofilm mass developed by the bacterial cultures on different surface coupons was quantified using the crystal violet binding assay [12]. The amount of biofilm accumulated on each surface coupon was stained with a 2% crystal violet solution, followed by water rinsing. The dye in stained biofilm was extracted using an ethanol–acetone solution (80:20). The biofilm mass on each surface coupon was expressed by the amount of crystal violet dye (OD_{550}) released from stained biofilms. The OD_{550} values of the control samples were deducted from those of the tested samples.

2.4. Biofilm Removal Using Sanitizer Treatments

Sodium hypochlorite (pH 7.0, 200 ppm; Fisher Scientific), peracetic acid (130 ppm; Spartan Chemical Company, Inc., Maumee, OH, USA), Alpet D2 (IPAQuat; 58.6% isopropanol alcohol, 150 ppm quaternary ammonium compound; Best Sanitizers Inc., Penn Valley, CA, USA), and commercial liquid dish soap (0.5%; Dawn [13], Proctor & Gamble, Cincinnati, OH, USA) were selected for the study. The sodium hypochlorite and peracetic acid were selected based on the results of an informal survey among blueberry growers in the Pacific Northwest. The alcohol-based sanitizer and soapy water were recommended by a food safety expert working for the blueberry industry, as some growers have used them to clean and sanitize berry harvest containers, and perhaps the surface of OTR machine harvesters.

The surface coupons with developed biofilms were rinsed with 5 mL of sterile deionized water to remove bacterial cells that are loosely associated with the surface coupons. Washed coupons were submerged in each of the four sanitizer solutions for 1 min at ambient temperature. Spent sanitizer solutions were discarded, and treated coupons were immersed in sterile Dey-Engley neutralizing broth (Becton, Dickinson, and Company) for 10 min to neutralize the sanitizers, followed by washing with deionized water for 5 s. The residual biofilm mass on sanitized coupons was quantified using the crystal violet binding assay described above.

2.5. Statistical Analysis

Each experiment was repeated twice, and all samples had duplicates in each set of experiments. Data from the experiments were fitted into the generalized linear mixed model of SAS OnDemand for Academics (SAS Institute, Cary, NC, USA), and Fisher's least significant difference test was used to separate the means. *p*-values smaller than or equal to 0.05 were considered significantly different.

3. Results

3.1. Characteristics of the Bacterial Isolates Used in the Study

Results of 16S rDNA sequencing revealed that the nine biochemically confirmed fecal coliform isolates used in the study were all *E. coli*. The six virulence genes of diarrheagenic *E. coli* specified in Table 2 were not detected in eight out of the nine isolates used in the study. However, 177, one of the isolates in culture mix V previously isolated from the surface of an OTR machine harvester, tested positive for *elt*, the gene that encodes for the *E. coli* heat-labile enterotoxin. This result was confirmed by Sanger DNA sequencing.

E. coli isolates 177 and 178 in culture mix V and isolate 1212 in culture I each tested positive for nine putative adhesin genes (Table 3). Isolate 1238, 1243, and 2470 in culture mix II tested positive for nine, seven, and six putative adhesin genes, respectively. The single isolate in culture IV, 2561, was positive for five putative adhesin genes, whereas isolates 2901 and 2902 in culture mix III carried six and five putative adhesin genes, respectively. All nine isolates tested positive for loc 3, 5, 7, and 14, while none of them tested positive for loc 9.

Table 3. Presence of putative adhesin genes in the *E. coli* isolates used in the study.

	Putative Adhesin Genes													
	***stc*A**	***yad*N**	***sfm*A**	***ybg*D**	***ycb*Q**	***sfa*A**	***csg*A**	***fim*A**	***yeh*D**	***stf*G**	***yra*H**	***lpf*A**	***lpf*A**	***fim*A**
177	+	+	+	+	+	+	+	-	-	+	-	-	-	+
178	+	+	+	+	+	-	+	-	-	+	+	-	-	+
1212	-	-	+	-	+	-	+	+	-	+	+	+	+	+
1238	+	-	+	+	+	+	+	+	-	+	-	-	-	+
1243	-	-	+	-	+	-	+	+	-	+	+	-	-	+
2470	-	-	+	-	+	-	+	+	-	-	+	-	-	+
2561	-	-	+	-	+	-	+	-	-	-	+	-	-	+
2901	-	-	+	+	+	-	+	-	-	-	+	-	-	+
2902	-	-	+	+	+	-	+	-	-	-	-	-	-	+
%	33.3	22.2	100.0	55.6	100.0	22.2	100.0	44.4	0.0	55.6	66.7	11.1	11.1	100.0

3.2. Biofilm Formation on Surface Coupons

Results of the type III tests revealed that surface coupon material was a significant ($p \leq 0.05$) factor, while the *E. coli* culture used in the study was an insignificant ($p > 0.05$) factor influencing the formation of biofilms on tested coupon materials (Table 4). There was no significant interaction between the two variables. The two silicon, plexiglass, and neoprene coupons had similar, or numerically different, amounts of biofilm mass which were significantly lower than the biofilm mass on the EPDM coupons (Figure 1). Culture V and culture mix II accumulated similar amounts of biofilms which were significantly lower than the biofilms formed by culture mix V. The biofilm formed by culture I and culture mix III was not significantly different from those formed by other cultures used in the study.

Table 4. Results of type III tests for the biofilm formation experiments.

Source	Num DF	Den DF	F Value	Pr > F
Surface	4	48	8.97	<0.0001
Culture	4	48	1.82	0.1413
Surface*culture	16	48	0.86	0.6203

Num DF: number of degrees of freedom. Pr > F: *p*-value, reflects the significance of the effect; $p \leq 0.05$ is a significant effect.

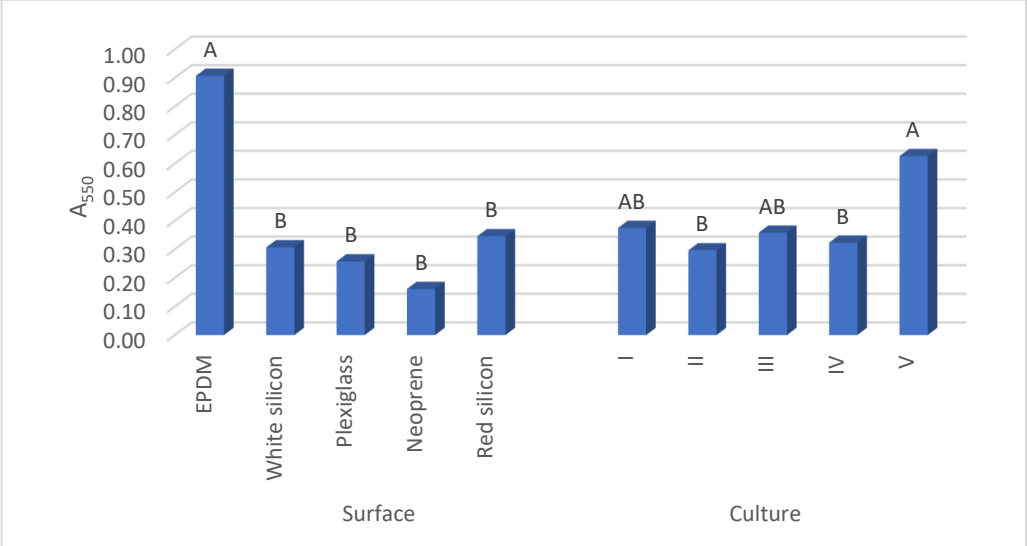

Figure 1. Overall mean biofilm mass formed on various surface coupons and by individual *E. coli* cultures or culture mixes. Bars of the same variables (surface coupons or bacterial cultures/culture mixes) with different letters were significantly different ($p \leq 0.05$). EPDM: ethylene propylene diene monomer.

3.3. Biofilm Control

Results of statistical analysis revealed that the *E. coli* culture, surface coupon material, and chemical treatment used in the study were all significant ($p \leq 0.05$) factors influencing the efficacy of biofilm removal, and there was a significant interaction between coupon material and *E. coli* culture, and between coupon material and sanitizer treatment (Table 5). The red silicon coupons had significantly less, while EPDM coupons had significantly more biofilm residues than plexiglass coupons after the sanitizer treatments (Figure 2). The biofilm residues on the neoprene and white silicone coupons were not significantly ($p > 0.05$) different from the residue on the plexiglass coupons. On average, EPDM coupons had the highest biofilm residues among all evaluated materials. Residues of biofilms formed by culture/culture mix III, IV, and V were similar, and significantly lower than the residues of biofilms formed by culture I and culture mix II. Treatments with dish soap removed significantly more biofilms than those with NaOCl and PAA. The efficacy of the Alphet D2 treatment was similar to the efficacies of the other three treatments used in the study. On average, the treatments with NaOCl and PAA both removed ca. 56% of the biofilm mass from the five types of coupons, while the treatment with Alphet D2 and soapy water removed 66% and 76% of the biofilm mass, respectively.

Table 5. Results of the type III test for the sanitation experiment.

	Num DF	Den DF	F Value	Pr > F
Surface	4	147	26.61	<0.0001
Treatment	3	147	4.83	0.0031
Culture	4	147	35.88	<0.0001
Surface*treatment	12	147	5.71	<0.0001
Surface*culture	16	147	5.60	<0.0001
Treatment*culture	12	147	1.26	0.2484

Num DF: number of degrees of freedom. Pr > F: *p*-value, reflects the significance of the effect; $p \leq 0.05$ are significant effects.

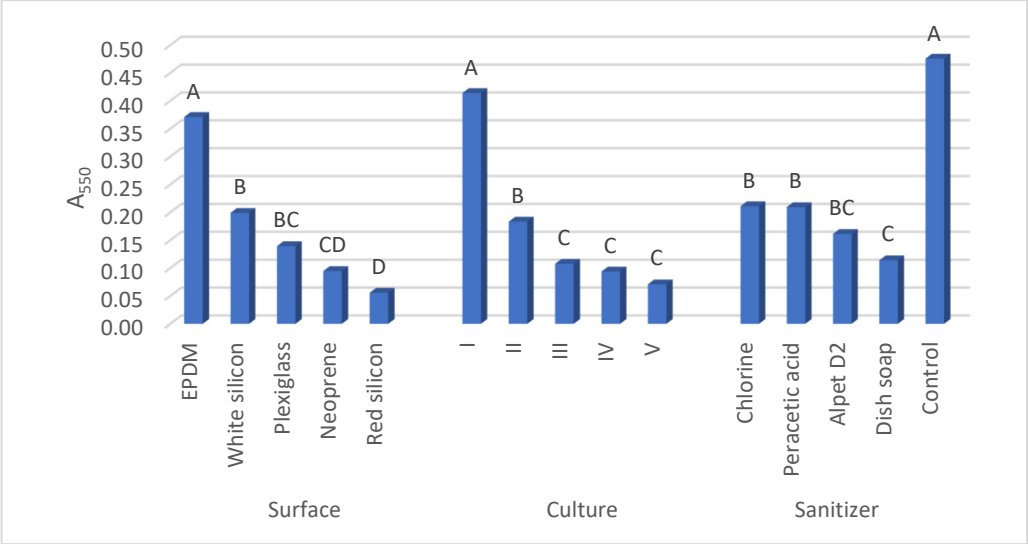

Figure 2. Overall mean residual biofilm mass of each *E. coli* culture or culture mix, on various coupon surfaces, and after the sanitizer treatments. Bars of the same variables (surface coupons, bacterial cultures/culture mixtures, or sanitizers used) with different letters were significantly different ($p \leq 0.05$). EPDM: ethylene propylene diene monomer.

Residues of biofilms formed by individual culture/culture mix left on each type of surface coupon after all four types of sanitizing treatments are summarized in Table 6. EPDM coupons had the greatest amounts ($p \leq 0.05$) of biofilm residues compared to

other coupons used in the study except for culture mix V and III on plexiglass, and culture IV on neoprene coupons. On coupons other than EPDM, the biofilm residues were essentially similar ($p > 0.05$) except for the biofilm residues of culture I. In comparison, the culture I biofilms left the greatest amounts of residues compared to other cultures on EPDM, plexiglass, and white silicone coupons. On red silicone coupons, however, the five cultures/culture mixes used in the study had similar amounts of biofilm residues. The biofilm residue of culture mix V on neoprene coupons was similar to the residues of biofilms formed by culture/culture mix II, III, and IV, and it was only significantly lower than the residue of culture I biofilms.

Table 6. Residues of biofilms of individual *E. coli* cultures or culture mixes, left on each surface coupon after all four types of sanitizing treatments.

	Biofilm Mass (A_{550})				
Bacterial Cultures or Culture Mixes	**I**	**II**	**III**	**IV**	**V**
Surface coupons					
EPDM (n = 8)	0.8971 Aa	0.3877 Ab	0.2421 Abc	0.2060 Ac	0.1224 Ac
Plexiglass (n = 8)	0.3922 Ca	0.1342 Bb	0.1053 ABb	0.0551 Bb	0.0098 Ab
Neoprene (n = 8)	0.1789 Da	0.1259 Bab	0.0797 Bab	0.0817 ABab	0.0073 Ab
Red silicone (n = 8)	0.0619 Da	0.1288 Ba	0.0316 Ba	0.0216 Ba	0.0910 Aa
White Silicone (n = 8)	0.5399 Ba	0.1463 Bb	0.0801 Bb	0.1050 ABb	0.1244 Ab

Means followed by the uppercase letters compare the significance of the differences ($p \leq 0.05$) in residual biofilm mass on different coupon surfaces. Means followed by the lowercase letters show the significance of the differences ($p \leq 0.05$) in residual biofilm mass formed by different *E. coli* cultures or culture mixes. EPDM: ethylene propylene diene monomer.

The residual biofilm mass left by all tested cultures/culture mixtures on each type of coupon after individual sanitizer treatment is summarized in Table 7. Biofilm residues on coupons other than those of EPDM treated with NaOCl and PAA were statistically similar ($p > 0.05$), and these residues were significantly ($p \leq 0.05$) lower than the biofilm mass left on the EPDM coupons. Furthermore, biofilm residues on all five types of coupons treated with dish soap were not significantly different from one another. Alphet D2-treated plexiglass, red silicone, and neoprene coupons had similar amounts of biofilm residues which were significantly lower than those on white silicone coupons. The lowest residual biofilm on coupons treated by Alphet D2 was observed on the red silicone coupons, which were significantly lower than the residues on the white silicon and EPDM coupons. The efficacies of all four sanitizer treatments were similar on plexiglass, neoprene, and red silicone coupons (Table 7). On white silicone coupons, the treatments with PAA and dish soap removed more biofilm residues than the treatment with Alphet D2. Furthermore, EPDM coupons treated with NaOCl and PAA had significantly more biofilm residues than those treated by the other two sanitizers used in the study.

Table 7. Average biofilm residues left by all cultures/mixtures on individual surface coupons after each sanitizer treatment.

	Biofilm Residue ($_{A500}$)			
Treatment	**NaOCl**	**Peracetic Acid**	**Alphet D2**	**Dish Soap**
Surface coupons				
EPDM (n = 8)	0.5886 Aa	0.5453 Aa	0.2293 ABb	0.1211 Ab
Plexiglass (n = 8)	0.0724 Ba	0.1885 Ba	0.1249 BCa	0.1714 Aa
Neoprene (n = 8)	0.1329 Ba	0.0711 Ba	0.1066 BCa	0.0682 Aa
Red silicone (n = 8)	0.0693 Ba	0.0826 Ba	0.0590 Ca	0.0524 Aa
White silicone (n = 8)	0.1920 Ba	0.1600 Ba	0.2874 Aa	0.1571 Aa

Means followed by the uppercase letters compare the significance of the differences ($p \leq 0.05$) in residual biofilm mass on different coupon surfaces. Means followed by the lowercase letters show the significance of the differences ($p \leq 0.05$) in residues of biofilm mass formed by different *E. coli* cultures or culture mixes. EPDM: ethylene propylene diene monomer.

4. Discussion

4.1. Biofilm Formation on Different Types of Coupons

Results of the study showed that EPDM coupons had significantly ($p \leq 0.05$) more biofilm mass, and the red and white silicone coupons had numerically ($p > 0.05$) more biofilm mass than did plexiglass and neoprene coupons (Figure 1). This result could be partially attributed to the surface energies of evaluated materials. According to the available information in the literature, neoprene and plexiglass have similar surface energies, being 3.9×10^{-2} to 4.1×10^{-2} N/m and 4.1×10^{-2} N/m, respectively [14,15]. However, the surface energy of EPDM is 2.8×10^{-2} N/m, and that of silicone is from 1.9×10^{-2} to 2.2×10^{-2} N/m [16], which are relatively lower compared to those of plexiglass and neoprene. Therefore, the hydrophobicity of EPDM and silicon is expected to be higher than that of neoprene and plexiglass. It is well-known that bacteria cells are negatively charged and tend to attach to materials that are relatively more hydrophobic with little surface energy or hydrophilic with positive charges [17].

However, it is important to point out that surface energy and hydrophobicity are not the only determining factors for attracting bacterial cells to their contact surfaces; other factors such as surface topography, surface roughness, and surface waviness could also be influencing factors [18]. For instance, a surface with higher roughness provides a larger surface area for bacterial attachment and adhesion, ultimately forming more biofilms [19]. Surface porosity and other surface conditions could also affect the attachment of, and biofilm formation by, bacterial cells [17,20].

While biofilm formation on the surface of EPDM, neoprene, and silicone has been assessed individually, studies on the relative amounts of biofilm mass built on the three types of surfaces are scarce. Maile [21] investigated alternative materials to silicone rubber for reducing *Candida albicans* biofilm formation in in-dwelling urinary catheters and found there was no significant difference in the biofilm levels formed on silicone vs. neoprene discs, a finding similar to the observation of the current study (Figure 1). Different from the results of our study, Hutchins et al. [22] found no difference in the biofilm mass formed by *Pseudomonas* on EPDM and silicone surfaces after 12 weeks in an experimental water distribution system. In the current study, a modified crystal violet assay was used to assess the biofilm mass on each surface, whereas, in the study of Hutchins et al., the amount of biofilm mass was determined by cell culture and microscopy.

4.2. The Effects of Culture on Biofilm Formation

Among the five sets of *E. coli* cultures used in the study, culture mix V, on average, formed significantly ($p \leq 0.05$) more biofilms on coupon surfaces (Figure 1). The two isolates in culture mix V were previously isolated from the surface of an OTR machine harvester, and they have probably adapted to the harsh environments in blueberry fields and become strong survivors and proliferate growers. Furthermore, both isolates carried nine out of the fourteen putative adhesin genes screened in the study (Table 3), and these surface structures may have provided them with a strong ability to interact with surface coupons. Isolate 1212 in culture I also carried nine putative adhesin genes, and the amount of biofilm formed by this isolate was statistically similar ($p > 0.05$) to that produced by culture mix V (Table 3 and Figure 1). However, the production of adhesins confers only part of cells' ability to interact with surface materials. Several other intrinsic factors such as the expression of flagella and polysaccharides could also come into play [23,24]. This could explain why isolates 2901 and 2902 in culture mix III produced a similar amount of biofilm mass compared to culture I and culture mix V by carrying only six and five putative adhesin genes, respectively (Table 3 and Figure 1).

Among fecal coliforms isolated from fresh produce packing environments, *E. coli* only makes up a small fraction of it [25]. The presence of *E. coli* isolates carrying virulence genes is even scarcer. However, 177, previously isolated from the surface of an OTR machine harvester, carried *elt*, the gene of *E. coli* heat-labile enterotoxin. This finding highlights the

importance of maintaining environmental hygiene and controlling cross-contamination between harvest equipment and fresh market blueberries.

4.3. Biofilm Removal from Different Coupons

As shown by the biofilm study, all five cultures/mixtures used in the study accumulated the greatest amount of biofilm mass on EPDM coupons (Figure 1); the surface was consistently found to have the most bioburdens for biofilm removal (Figure 2). On the contrary, the other materials had relatively lower biofilm buildups compared to EPDM, and thus lower amounts of biofilm residues after the sanitizer treatments. On average, culture mix V formed the greatest amount of biofilms on coupons (Figure 1), but had the lowest biofilm residue after the sanitizer treatments (Figure 2). In addition, culture mix II formed the lowest amount of biofilms but had a post-sanitation biofilm residue that was only lower than that of culture I (Figures 1 and 2). These observations suggest that both the quantity and quality of the biofilms may affect their susceptibility to sanitizer treatments.

Results of the present study suggest that neoprene and perhaps silicon are more promising materials from a microbiological and food safety perspective than EPDM for the modification of the plexiglass fruit-catching plates of OTR machine harvesters. However, food hygiene and food safety are not the only considerations for selecting suitable materials for the modification of fruit-catching plates. Material cost and durability, ease of fabrication, and user acceptability should also be among the considerations of harvester manufacturers.

4.4. Interaction between Sanitizer Treatment and Type of Surface Coupons

Results of the research showed that, on average, treatments with commercial dish soap removed significantly ($p \leq 0.05$) more biofilms than treatments with NaOCl and PAA (Figure 2). Chlorine is a potent chemical sanitizer but is sensitive to temperature, pH, and the presence of organic materials [26]. Peracetic acid can form hydrogen peroxide in solutions, inactivating microorganisms on surface coupons [27]. The dish soap contains several surfactants, cleaning agents, antibacterial agents, solvents, and water softeners [13], and the hurdle effect of these chemical ingredients might have made the dish soap more effective in removing the biofilm mass from the surface coupons used in the current study.

5. Conclusions

The study suggests that it is imperative to keep the food safety perspective in mind when selecting substitute materials for plexiglass, considering that some microorganisms that have the potential to colonize machine harvester surfaces may carry virulence genes. Among the types of surface materials evaluated in the study, neoprene and perhaps silicon seem to have more potential, from a microbiological standpoint, than EPDM to modify the plexiglass fruit-catching plates of the OTR machine harvester. However, these results should be further evaluated by harvester manufacturers to determine their suitability from the standpoints of mechanic fabrication, material durability, and economic feasibility. In addition, the surface characteristics of industry-use elastomeric polymer sheets may slightly vary from batch to batch, which may affect the level of biofilm buildup and efficacy of sanitizer treatments. Thus, additional batches of materials manufactured or distributed by different suppliers should be further evaluated. In the United States, chlorinated water and peracetic acid are more commonly used for sanitizing machine harvesters. Based on the results of the current study, liquid dish soap could be incorporated into sanitation regimens for surfaces that have a greater potential for biofilm formation.

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