Why Are Wet Wipes So Difficult to Preserve? Understanding the Intrinsic Causes

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Abstract: Over the last two decades, significant advances have been made in developing disposable baby wet wipes. Wet wipes consist of two main components: nonwoven fabric and liquid. Being more than 90% water, wet wipes are more susceptible to microbial growth than typical personal care products; hence, high concentrations of preservative compounds are often used to ensure extended protection against contamination. However, there is an obvious tendency to minimize the concentration of irritating actives. Baby wet wipes should contain particularly mild surfactants, well-tolerated preservatives, and a buffer system maintaining the formulation pH at a suitable level for the infant’s skin. Efforts have been centered on removing ingredients with irritation potential, such as phenoxyethanol. In addition, a move towards more natural fabrics is occurring. However, these modifications provoke new challenges in preserving the final products. The nature and composition of the fiber can influence the interactions between the preservative and the wipe, subsequently affecting the performance of the preservative system. In this study, we analyzed the causes of the challenge in preserving wet wipes. We found that fabrics containing natural fibers are the main source of contamination, promoting the generation of biofilms on their surfaces. Moreover, the hydrophilic–lipophilic balance (HLB) was utilized to rationalize the physicochemical interactions between the fabric and the preservatives.

Keywords: wet wipes; preservation; biofilm; hydrophilic–lipophilic balance

1. Introduction

Wet wipes were originally developed as personal care products, yet with time they have become an increasingly popular item for everyday use in homes. Generally, wet wipes consist of two main parts: a nonwoven fabric carrier (wipe) and liquid. The wipes hold and spread the liquid or collect and hold dirt or other matters to be removed. The liquid impregnating the wipes is mostly water but also contains surfactants, a buffer, and preservatives. Nonwoven fabrics are broadly defined as sheet or web structures bonded together mechanically, thermally, or chemically by entangling fibers or filaments [1]. The fibers used to produce the wipe may be natural, such as wood pulp, or synthetic, such as polypropylene, polyester (PET), or combinations thereof. Low-cost synthetic fibers are the major raw materials of wet wipes, while natural fibers including wood pulp, viscose (a regenerated cellulose product), and cotton may be used for fine-tuning the desired features of the final product. The different compositions are generally translated into variations in thickness, absorbency, and softness.

Several methods exist to produce nonwoven fabrics, and in this study we used fabrics manufactured by spunlace technology. The resulting dimensional properties of the nonwoven fabric, including surface density, thickness, porosity (ratio of void volume to total fabric volume), pore size, and structure, would mainly affect the fiber strength, flexibility, absorbency, and liquid diffusion behavior. In general, higher volume porosity gives a higher vertical wicking rate, regardless of the content of the hydrophilic/hydrophobic fibers.
Various spunlace process parameters can have effects on the abovementioned fiber structural characteristics, such as the waterjet pressure, delivery speed, web mass, and web composition [3]. It has been suggested previously that some of the physicochemical properties of the fabric can affect bacterial adhesion to the surface [4–6].

Wet wipes contain a large amount of water (>90%) and are therefore susceptible to the growth of microbes [7–9]. Therefore, the water used in wet wipes ranges from highly purified to reverse osmosis quality. The water treatment process removes most of the salt content (calcium and magnesium carbonates, contributing to overall hardness) and other residual minerals that can serve as nutrients for microorganisms. In addition, many water systems employ ultraviolet light processing to sterilize the water before use. As a result, the water in wet wipes is of a higher quality than standard drinking water. Addition of common organic acids (malic acid or citric acid) and conjugate bases (sodium citrate, sodium bicarbonate, or sodium phosphate) is needed to adjust the pH of wet wipe formulations to match the pH of the skin surface [10]. A variety of anionic, cationic, and nonionic surfactants, which can adequately remove skin dirt, are used in the formulations. In the case of infant wet wipes, the surfactant fraction is very low (usually <1% w/w), which is in contrast to bottled baby products, such as body washes, shampoos, and hand soaps, where the surfactant concentration is typically up to 20% w/w [11].

The preservation of wet wipes often presents challenges that are not encountered with other personal care products due to the vast surface area of the nonwoven fabric, the high water content, and the formation of biofilms (microbial communities attached to surfaces) [12]. Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface, producing extracellular polymers that facilitate their attachment and the formation of matrices, resulting in an alteration of the microorganisms’ growth rate. Moreover, these biofilm-associated microorganisms exhibit a dramatically decreased susceptibility to antimicrobial agents [13].

Additionally, cellulose-based fibers can be degraded into glucose units that provide suitable nutrients for microbial colonization (biofilms). The biodegradability of cellulose-based materials for extracellular enzymes excreted by various microbes has been extensively studied [14–18]. Fungi, including *Trichoderma*, *Penicillium*, and *Fusarium* spp., are common, efficient producers of cellulolytic enzymes [19]. *B-glucosidase*, secreted from *Aspergillus brasiliensis*, is an essential enzyme for the complete hydrolysis of cellulose-based fibers [19]. A series of enzymatic processes (caused by microbes) has been found to result in the catabolization of sugar molecules to generate stored energy within adenosine triphosphate (ATP) [20,21].

Biofilms have been studied extensively over the past 20 years and much is known about the process of microbial attachment and initial biofilm formation [12,13]. The development of microbial biofilm is described as a dynamic process involving several steps. At first, bacterial cells attach to the surface through weak reversible van der Waals interactions, which can develop into stronger adhesion attachments, e.g., dipole, hydrogen, ionic, or hydrophobic [12]. The second step corresponds to the generation of microcolonies promoted by the growth and division of the first attached cells. The microcolonies progressively enlarge and coalesce to form the first layer of cells covering the surface. When multiple layers of cells pile up on the surface, the third step of the formation is obtained, indicated by the presence of a mature biofilm, highly hydrated (98% water) and tenaciously bound to the underlying surface. Their structure has been found to be heterogeneous, both in space and over time, with water channels that allow transport of essential nutrients and oxygen to the cells growing within the biofilm [22].

A widespread marketing trend, in which personal care products are marketed as free of controversial preservative ingredients, e.g., parabens and formaldehyde donors, is producing further difficulties in baby wet wipe preservation. Therefore, in this study we aimed to locate the origins of the preservation difficulties that can be controlled in order to design a rational and efficient wet wipe preservative system.
For this purpose, we examined the impact of fabric composition, using viscose, polyester, and their combination, on preservative efficacy in wet-wipe liquid formulations. Assessment of biofilm formation was performed on either polyester or viscose fibers, and the physicochemical characteristics of various preservatives were evaluated in comparison with those of viscose and PET. These data made it possible to evaluate the compatibility and efficiency of the preservative molecules relative to the fabrics. Finally, we establish a clearer understanding of the origin of the difficulties in wet wipe preservation in order to provide the foundation for further development of novel antimicrobial strategies.

2. Materials and Methods

2.1. Materials

Benzoic acid (99.99% purity) was purchased from Emerald Kalama Chemical, (Kalama, WA, USA). Sorbic acid (99.3%) and potassium sorbate (99.2%) were bought from Nantong Acetic Acid Chemical Co. Ltd. (Nantong, China). Methylparaben (>99.86%) was purchased from Zhejiang Sheng Xiao Chemical Co. Ltd. (Quzhou, China) and sodium benzoate (99.7%) from Wuhan Youji Industries Co. Ltd. (Wuhan, China). TDMDMH (55%) was from Sharon Laboratories Ltd., (Ashdod, Israel). The PET, viscose, and the PET–viscose combination for wipes were kindly provided by N.R. Spuntech Industries Ltd. (Tiberias, Israel). All fibers were produced by spunlace technology, also known as the hydroentangling process, in which a series of high velocity, very fine water jets are utilized to mechanically interlock and entangle fibers. All the tested nonwoven wet wipes were produced with a highly repeatable process.

2.2. Formulation

The following liquid wet wipe formulation was used in challenge tests to evaluate the origin of the contamination of wet wipes (Table 1).

Table 1. Wet wipe liquid formulation tested by challenge test.

| Trade Name | INCI           | Function               | % Weight |
|------------|----------------|------------------------|----------|
| Water      | Aqua           | Solvent                | 98.05%   |
| Chamomile extract | Chamomile ext. | Humectant              | 0.05%    |
| Panthenol  | Panthenol      | Skin conditioning      | 0.05%    |
| Glycerin   | Glycerin       | Humectant              | 0.50%    |
| Sodium citrate | Sodium citrate |                       | 0.05%    |
| Tween 20   | Polysorbate 20 | Surfactant (nonionic)  | 0.60%    |
| Preservative | Tocopherol    | Preservative           | 0.45     |
| Vitamin E  | Disodium cocoamphodiacetate | Surfactant (anionic) | 0.20%    |

All ingredients were purchased from Ziv Chemicals (Holon, Israel), with the exception of sodium citrate (99 > 0%) which was bought from Sigma Aldrich (Rehovot, Israel). In some cases, challenge tests were done using saline only as the aqueous phase (Tables 2 and 3) in order to eliminate any parameters that might affect the results, such as surfactants.

2.3. Challenge Test

The microorganisms’ strains and growth conditions have been described previously [23]. Challenge tests for preservative efficacy were performed according to ISO 11930 regulations. Wipe fibers were impregnated in a ratio of 1/4 (w/v) with the formulation described in Table 1, at a pH of 5.5, and inoculated separately in plastic bags with each microorganism (Escherichia coli ATCC 8739, Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC
Candida albicans ATCC 10231, and Aspergillus brasiliensis ATCC 16404), with final concentrations of $10^{5}–10^{6}$ CFU/mL for bacteria and $10^{4}–10^{5}$ for yeast and mold. Wet wipes were homogenized thoroughly using a Stomacher STO-4 paddle blender (MRC, Holon, Israel) and were sealed and incubated in the dark at 22 °C for 28 days. The preservative efficacy was determined by sampling one wet wipe at time points of 2, 7, 14, 21, and 28 days. To enumerate the microorganisms at each time point, liquid was added to the bags to create serial dilutions up to $10^{-4}$, and 1 mL was seeded onto a petri dish with the appropriate media, TSA/SDA (bacteria vs. yeast respectively), using the pour plate method. Plates were incubated at 32 °C for 3 days for bacteria, while yeast and mold were incubated at 22 °C for 5 days until the enumeration of viable microorganisms. Evaluation of the preservative efficacy was undertaken according to the United States Pharmacopoeia (USP) standards [24]. The efficacy of the preservative, for there were 100-fold and 1000-fold reductions from the initial inoculated bacterial count at days 2 and 7, respectively, was considered adequate according to the EP acceptance criteria. Moreover, according to previous studies, no increase in bacterial count should appear between day 14 and day 28 [25–27]. Yeast and mold should show 100-fold reductions after 14 days with no further increase in the following weeks [25–27].

2.4. Scanning Electron Microscopy (SEM)

Samples were fixed with Karnovsky’s fixative (2% PFA, 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH = 7.4) for 4 h at room temperature, followed by 1/2 diluted Karnovsky’s fixative overnight at 4 °C. Samples were then placed on a coverslip (coated with 0.1% of poly-l-lysine), post-fixed in 1% osmium tetroxide (OsO$_4$) in 0.1 M cacodylate buffer for 2 h, and dehydrated in a graded series of alcohols. Then, samples were dried in a CPD (Quorum Technology K850, Lewes, UK) and coated with Pd/Au (Quorum Technology SC7620, UK).

Samples were imaged in a Quanta 200 Scanning Electron Microscope (Hebrew University Jerusalem, Jerusalem, Israel). Images were collected with a 1M pixel camera and the acquisition time per image was 60 s. This part of the work was done by the EM Unit of the Core Research Facility of the Faculty of Medicine, Hebrew University Jerusalem (Dr. Eduard Berenshtein).

2.5. Hydrophilic–Lipophilic Balance (HLB) Calculation

Griffin’s hydrophilic–lipophilic balance (HLB) formula (Formula (1)) was utilized, derived from the non-ionic surfactant field [28,29]. According to Griffin, the HLB of an amphiphilic molecule is the value obtained by dividing the weight fraction of the hydrophilic part by the total weight of the molecule and multiplying by twenty, giving a result on a scale of 0 to 20. An HLB value of 0 corresponds to a highly hydrophobic molecule while a value of 20 corresponds to a highly hydrophilic one. By comparing the HLB values of diverse preservative molecules, as well as those of viscose and PET (under their monomeric forms), we aimed to evaluate the affinity between the preservatives and the fabric constituents.

3. Results and Discussion

3.1. Origin of Contamination during Storage and Use

In order to identify the origin of wet wipe contamination, the liquid phase of wet wipes containing 0.45% aqueous preservative mixture, composed of 0.3% sodium benzoate and 0.15% potassium sorbate in the final wet wipe formulation, was examined with challenge tests in the presence and absence of nonwoven fabric. The composition of the liquid formulation was as described in Table 1. Figure 1 displays the susceptibility of E. coli, S. aureus, P. aeruginosa, C. albicans, and A. brasiliensis microorganisms to 0.45% organic acids in the two wet-wipe liquid systems: the wet-wipe liquid without the introduction of the fabric and the wet-wipe liquid that had been in contact with 80/20 PET/viscose nonwoven fabric. The challenge results demonstrated that, when the nonwoven wipes were absent
(Figure 1A), the challenge test met the EP test criteria, while in the presence of the 80/20 PET/viscose fabric, it did not (Figure 1B). This suggests that the fabric, and/or interactions that may arise due to its existence, contributes to the contamination of the wet-wipe liquid system.

![Graph A](image1.png)

**A** No fabric

![Graph B](image2.png)

**B** With fabric

![Figure 1.](image3.png)

**Figure 1.** Challenge test of organic acid preservative mixture (0.45%) composed of 0.3% sodium benzoate and 0.15% potassium sorbate: (A) in the absence of wipe fabric and (B) in its presence. Data are presented as log$_{10}$ of CFU/mL.

To clarify which part of the fabric was responsible for the contamination and to what extent, we examined the efficacy of the same wet-wipe liquid system containing the preservatives when it was combined with four different types of fabrics: 100% PET, 90/10% PET/viscose, 80/20% PET/viscose, and 100% viscose. Fabrics containing the wet-wipe liquid system were incubated separately with two selected microbes, *E. coli* and *P. aeruginosa*, and as presented in Figure 2.
3.2. Biofilms Formation on Viscose and PET Nonwoven Fabrics

In this study, SEM was used to detect biofilms following three hours’ incubation with $10^6$ CFU/mL of *P. aeruginosa*, *E. coli*, and *S. aureus* microorganisms, separately, on viscose vs. PET fibers (Figure 3). SEM images of the viscose fiber showed large bacterial colonies presenting as biofilms, while the PET fiber surfaces appeared rather “clean”. It is well-known that the extracellular polymers (i.e., biofilms) produced by microbial colonies primarily consist of polysaccharides [13]; therefore, it is reasonable to assume that these polysaccharides could easily adhere to the polysaccharidic cellulosic fabric. As a consequence, biofilms would indeed favorably form on the viscose surface rather than on the PET one, having a limited affinity for the latter. These observations agree with and confirm the outcome of the challenge tests (Figure 2), stressing that the viscose fabric bears the key role in wet wipe contamination.

**Figure 2.** Challenge tests of four combinations of fabrics: 100% PET, 90/10% PET/viscose, 80/20% PET/viscose, and 100% viscose—impregnated with a preservative system containing a 0.45% organic acid mixture following 2 days’ incubation with *E. coli* and *P. aeruginosa*. Data are presented as $\log_{10}$ of CFU/mL.

A comparison between 100% PET and 100% viscose revealed that the presence of the 100% viscose fabric led to an increase in microorganisms’ growth relative to the inoculum level, while in the presence of 100% PET the microbial level decreased. Surprisingly, the presence of 10%, 20%, or 100% viscose did not considerably affect the bacterial count, suggesting that even modest cellulose content contributed to the growth.

The mean pore diameter size of the 100% PET nonwoven has previously been found to be higher than that of 100% viscose nonwoven fabrics with the same waterjet pressure [3]. When viscose fibers are added to PET, such as in Figure 2, the mean pore size decreases gradually in comparison to 100% PET [3]. The pore volume and the pore size are important factors affecting bacterial adherence and retention [6]. Bacteria prefer to adhere to the gaps between fibers and inside pores; therefore, smaller gaps between fibers, or smaller pore sizes, which subsequently lead to a smaller total pore volume, are correlated with decreased bacterial adhesion [4,6]. These observations from previous studies are in contradiction with our results in Figure 2, where increased percentages of viscose fibers, which had a lower mean pore size, caused an increase in bacterial growth in comparison to 100% PET fibers. This emphasizes the importance of the chemical factor, represented by the differences in the polarity of the viscose and PET fibers, which overrides the influence of the physical one, represented by the pore size.
3.3. HLB Calculations to Evaluate Preservative/Fabric Compatibility

A wide variety of preservatives are available in the cosmetics market, but a suitable molecule needs to be chosen to provide maximum efficacy in the final product. In order to determine a predictive compatibility between the nonwoven molecular structure(s) and the preservative molecule(s), Griffin’s hydrophilic–lipophilic balance (HLB) formula (Formula (1)), was utilized.

\[
\text{HLB} = 20 \cdot \frac{\text{Mw Hydrophilic}}{\text{Total Mw}}
\]

As expected, and as depicted in Figure 4, the HLB value of the PET fabric was lower than the viscose one. Strikingly, it was found that all the examined preservative molecules had lower HLB values than the PET HLB, indicating that they were even more hydrophobic than the most hydrophobic fabric fiber (PET). Therefore, we may conclude that most commonly used preservatives in the wet wipes industry (i.e., organic acids, medium chain glycols, phenoxyethanol, parabens) will have a higher physicochemical affinity for hydrophobic fabrics (i.e., PET, polyethylene), and a lower affinity for hydrophilic fabrics, such as viscose and other cellulosic materials. When it is taken into consideration that these preservatives also display a limited solubility in water—for example, the organic acid mixture, used in the tests from Figures 1 and 2, was only 6.4% soluble in water at a pH of 5.5 with a log P of 0.13 for the active acid form—it is easy to understand that these preservatives are better designed to preserve hydrophobic fabrics, with which they might establish physicochemical interactions, thus leaving the hydrophilic fabrics and the aqueous medium inadequately protected.
Furthermore, previous studies have shown that bacterial adhesion decreases as the contact angle of a surface increases; hence, there is lower wettability and a more hydrophobic surface [5,6]. As the microorganisms were incubated in a hydrophilic matrix, they would have had a tendency to interact with the hydrophilic surface.

To verify the differential affinities between the preservatives and the fabrics and their relationships with the preservation efficacy, the following experiments were carried out. In a first set, two challenge tests were conducted in parallel (Table 2), comparing saline solutions with and without 100% PET fabric, with both solutions preserved with 0.2% methylparaben. Methylparaben, with an HBL value of 8 (Figure 4), has a low solubility of 0.25% in water, with a high log P of 1.96. An immediate reduction in microbial populations was observed in the saline treatment without the fiber, while in the presence of PET the antimicrobial activity was negligible. These results suggest that the high affinity of the hydrophobic preservative, methylparaben, with the hydrophobic PET fabric led to a lack of antimicrobial protection in the aqueous solution.

Table 2. Challenge test of 0.2% methylparaben in saline in the absence vs. presence of 100% PET fabric.

| Treatment                        | Time of Reading (Days) | E. coli (cfu/mL) | S. aureus (cfu/mL) | P. aeruginosa (cfu/mL) | C. albicans (cfu/mL) | A. brasiliensis (cfu/mL) |
|----------------------------------|------------------------|------------------|--------------------|------------------------|----------------------|------------------------|
| Saline containing 0.2% methylparaben without fabric | 2                     | $1 \times 10^6$ | $1.1 \times 10^6$ | $1 \times 10^6$       | $1 \times 10^5$      | $1.1 \times 10^5$      |
|                                  | 7                      | $<10$            | $<10$              | $<10$                 | $<10$                | $<10$                |
|                                  | 14                     | $<10$            | $<10$              | $<10$                 | $<10$                | $<10$                |
|                                  | 21                     | $<10$            | $<10$              | $<10$                 | $<10$                | $<10$                |
|                                  | 28                     | $<10$            | $<10$              | $<10$                 | $<10$                | $<10$                |
| Saline containing 0.2% methylparaben with 100% PET fabric | 2                     | $1 \times 10^6$ | $1.1 \times 10^6$ | $1 \times 10^6$       | $1 \times 10^5$      | $1.1 \times 10^5$      |
|                                  | 7                      | $3 \times 10^3$  | $1 \times 10^5$    | $4.1 \times 10^5$     | $1 \times 10^4$      | $1 \times 10^5$      |
|                                  | 14                     | $3 \times 10^3$  | $5 \times 10^3$    | $5 \times 10^5$       | $3 \times 10^2$      | $6 \times 10^4$      |
|                                  | 21                     | $1 \times 10^4$  | $5 \times 10^4$    | $10^1$                | $6 \times 10^4$      | $3 \times 10^3$      |
|                                  | 28                     | $3 \times 10^3$  | $6 \times 10^5$    | $10^1$                | $6 \times 10^4$      | $3 \times 10^3$      |

* TNTC: Too numerous to count.
To further verify this concept, a second set of two challenge tests were conducted in parallel (Table 3), comparing saline solutions with and without 100% PET fiber, with both solutions preserved with 0.5% DMDMH (55% active). The latter has a higher HLB value than the viscose (highly hydrophilic), >55% solubility in water, and a low log p value of −1.2; consequently, it was expected to have low affinity with the PET fabric. Indeed, these two challenge tests exhibited very similar antimicrobial performances, both when the PET fabric was present and absent. Hence, it is reasonable to assume that the affinity of the hydrophilic preservative with the PET was very low, giving the DMDMH antimicrobial potential to act within both aqueous solutions.

Table 3. Challenge test of 0.275% active DMDMH in saline in the absence vs. presence of 100% PET fabric.

| Treatment                  | Time of Reading (Days) | E. coli (cfu/mL) | S. aureus (cfu/mL) | P. aeruginosa (cfu/mL) | C. albicans (cfu/mL) | A. brasiliensis (cfu/mL) |
|----------------------------|------------------------|------------------|--------------------|------------------------|----------------------|-------------------------|
| Saline containing 0.275%   | 2                      | 1 × 10⁶          | 1.1 × 10⁶          | 1 × 10⁶                | 1 × 10⁵              | 1.1 × 10⁵               |
| without fabric             | 7                      | <10              | <10                | <10                    | <10                  | <10                     |
| DMDMH with 100% PET fabric | 14                     | <10              | <10                | <10                    | <10                  | <10                     |
| without fabric             | 21                     | <10              | <10                | <10                    | <10                  | <10                     |
|                            | 28                     | <10              | <10                | <10                    | <10                  | <10                     |

4. Conclusions

This study aimed to precisely identify the root causes of the inherent difficulty of preserving wet wipes from microbial contamination. To do so, various microbiological and physicochemical tools were used in a set of experiments to unveil the interactions existing between the nonwoven fabric constituents and commonly used preservatives. The results made it possible to rationalize the link between these interactions and the antimicrobial efficacy. Thus, the viscose hydrophilic material was found to be an ideal carrier for contamination, as was clearly presented by the SEM images of viscose fibers covered with biofilm structures in all three microorganisms tested. Its cellulosic material allowed it to be an intrinsic source of food for the microorganisms and promoted microbial colonization. Supporting these findings, increased bacterial growth was observed in preserved wet wipes that contained even a low amount of viscose (10%) in comparison to 100% PET fibers (Figure 2).

On the other hand, the PET hydrophobic material was found to be rather inert towards microorganisms, as could be observed in the SEM images. Nonetheless, it was evidenced that it shared a very significant indirect role in the contamination. Indeed, as observed by the challenge test using methylparaben (Table 2), a hydrophobic preservative with a rather low HBL value of 8 (Figure 4), when introducing the hydrophobic PET fiber to the aqueous phase, the preservative efficacy decreased significantly. As the vast majority of the preservatives currently used for wet wipes are hydrophobic, they will most likely develop an interaction with the hydrophobic nonwoven element, therefore leaving the hydrophilic cellulosic (viscose) material, along with the aqueous phase, inadequately protected and exposed to contamination. This observation was supported by the challenge test using DMDMH (Table 3), a hydrophilic preservative with a high HBL value of 15.5, where the preservative efficacy was maintained following the addition of the PET fiber, suggesting adequate protection of the aqueous phase.

We believe that the profound mismatch between the site of maximum preservation and the site of maximum contamination finds its cause in the inadequate relationships between
the natures of the preservative and nonwoven systems currently in use. Consequently, and in order to correct this situation, it is logical to consider an approach adapted towards a preservative system based on the association of a more hydrophilic nature and an antibiofilm capacity. We are currently dedicating our efforts on the development of such a preservative system, which will be published in a future article.

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References

1. Kaplan, S.; Pulan, S.; Ulusoy, S. Objective and subjective performance evaluations of wet wipes including herbal components. J. Ind. Text. 2018, 47, 1959–1978. [CrossRef]

2. Dubrovski, P.D.; Brezocnik, M. Porosity and nonwoven fabric vertical wicking rate. Fibers Polym. 2016, 17, 801–808. [CrossRef]

3. Jain, R.K.; Sinha, S.K.; Das, A. Structural investigation of spunlace nonwoven. Res. J. Text. Appar. 2018, 22, 158–179. [CrossRef]

4. Kargar, M.; Wang, J.; Lee, H.; Kim, J. Bacteria adhesion of textiles influenced by wettability and pore characteristics of fibrous substrates. Soft Matter 2012, 8, 10254–10259. [CrossRef]

5. Bajpai, V.; Dey, A.; Ghosh, S.; Bajpai, S.; Jha, M.K. Quantification of bacterial adherence on different textile fabrics. Int. Biodeterior. Biodegr. 2011, 65, 1169–1174. [CrossRef]

6. Hemmatian, T.; Lee, H.; Kim, J. Bacteria adhesion of textiles influenced by wettability and pore characteristics of fibrous substrates. Polymers 2021, 13, 223. [CrossRef] [PubMed]

7. Brown, A.D. Microbial water stress. Bacteriol. Rev. 1976, 40, 803–846. [CrossRef]

8. Manzoni, S.; Schimel, J.P.; Porporato, A.; Ecology, S.; April, N.; Manzoni, S.; Schimel, J.P.; Porporato, A. Responses of soil microbial communities to water stress: Results from a meta-analysis. Ecology 2012, 93, 930–938. [CrossRef] [PubMed]

9. Moyano, F.E.; Manzoni, S.; Chenu, C. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem. 2013, 59, 72–85. [CrossRef]

10. Rodriguez, K.J.; Cunningham, C.; Foxenberg, R.; Hoffman, D.; Vongsa, R. The science behind wet wipes for infant skin: Ingredient review, safety, and efficacy. Pediatr. Dermatol. 2020, 37, 447–454. [CrossRef]

11. Cunningham, C.; Mundschau, S.; Seidling, J.; Wenzel, S.; Maisen, G.D.N. The Chemistry and Manufacture of Cosmetics; Schlossman, M.L., Ed.; The Chemistry and Manufacture of Cosmetics; Allured Books: Chicago, IL, USA, 2008; ISBN 9781932633481.

12. Donlan, M.R. Biofilms: Microbial Life on Surfaces. Emerg. Infect. Dis. 2002, 8, 881–890. [CrossRef] [PubMed]

13. Donlan, R.M. Biofilm formation: A clinically relevant microbiological process. Clin. Infect. Dis. 2001, 33, 1387–1392. [CrossRef] [PubMed]

14. Hernández, M.; Hernández-Coronado, M.J.; Ball, A.S.; Arias, M.E. Degradation of alkali-lignin residues from solid-state fermentation of wheat straw by streptomycetes. Biodegradation 2001, 12, 219–223. [CrossRef] [PubMed]

15. Buschle-Diller, G.; Zeronian, S.H.; Pan, N.; Yoon, M.Y. Enzymatic Hydrolysis of Cotton, Linen, Ramie, and Viscose Rayon Fabrics. Text. Res. J. 1994, 64, 270–279. [CrossRef]

16. Wood, T.M. Fungal cellulases. Biochem. Soc. Trans. 1992, 20, 46–52. [CrossRef] [PubMed]

17. Wood, T.M.; García-Campayo, V. Enzymology of cellulose degradation. Biodegradation 1990, 1, 147–161. [CrossRef]

18. Dufour, D.; Leung, V.; Lévesque, C.M. Bacterial biofilm: Structure, function, and antimicrobial resistance. Endod. Top. 2010, 22, 2–16. [CrossRef]

19. Itavaara, M.; Vikari, L. Enzymatic Degradation of Cellulose-Based Materials. J. Environ. Polym. Degrad. 1999, 7, 67–73. [CrossRef]

20. Itiavaa, M.; Vikman, M. A Simple Screening Test for Studying the Biodegradability of Insoluble Polymers. Chemosphere 1995, 31, 4359–4373. [CrossRef]
22. Lewandowski, Z. *Biofilms: Recent Advances in Their Study and Control*; Evans, L.V., Ed.; Harwood Academic Publishers: Amsterdam, The Netherlands, 2000; P 1:2000; ISBN 9780203304723.

23. Ziklo, N.; Tzafrir, I.; Shulkin, R.; Salama, P. Salicylate UV-filters in sunscreen formulations compromise the preservative system efficacy against Pseudomonas aeruginosa and Burkholderia cepacia. *Cosmetics* **2020**, *7*, 63. [CrossRef]

24. Russell, A.D. Challenge testing: Principles and practice. *Int. J. Cosmet. Sci.* **2003**, *25*, 147–153. [CrossRef]

25. Joung, D.K.; Choi, S.H.; Kang, O.H.; Kim, S.B.; Mun, S.H.; Seo, Y.S.; Kang, D.H.; Gong, R.; Shin, D.W.; Kim, Y.C.; et al. Synergistic effects of oxyresveratrol in conjunction with antibiotics against methicillin-resistant Staphylococcus aureus. *Mol. Med. Rep.* **2015**, *12*, 663–667. [CrossRef]

26. Buffet-Bataillon, S.; Tattevin, P.; Bonnaure-Mallet, M.; Jolivet-Gougeon, A. Emergence of resistance to antibacterial agents: The role of quaternary ammonium compounds-A critical review. *Int. J. Antimicrob. Agents* **2012**, *39*, 381–389. [CrossRef]

27. Davidson, P.M.; Critzer, F.J.; Matthew Taylor, T. Naturally occurring antimicrobials for minimally processed foods. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 163–190. [CrossRef]

28. Griffin, W.C. Calculation of HLB values of non-ionic surfactants. *J. Soc. Cosmet. Chem.* **1954**, *5*, 249–256. [CrossRef]

29. Griffin, W.C. Classification of Surface-Active Agents by “HLB”. *J. Soc. Cosmet. Chem.* **1949**, *1*, 311–326.