

Article

Risk-Based Assessment of Cosmetic Preservation Using Raw-Material-Associated Microorganisms in an Adapted EN ISO 11930 Challenge Test

Binal Dobariya ¹, Ghazaleh Fazlikhani ¹, Marian E. Delsing ², Robert Beinio ² and Dirk P. Bockmühl ^{1,*} 

¹ Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, 47533 Kleve, Germany

² BBMED Product GmbH, Wöhrmannstr. 15, 47546 Kalkar, Germany

* Correspondence: dirk.bockmuehl@hsrw.eu; Tel.: +49-2821-806-73-208; Fax: +49-2821-806-73-44208

Abstract

Cosmetic formulations containing natural or minimally processed raw materials are prone to microbial contamination, which can compromise product safety and quality. The standard ISO 11930 preservation challenge test employs a predefined set of test microorganisms, which may not adequately represent product-associated contaminants. In the present study, an adapted ISO 11930 preservation challenge test was applied to compare the antimicrobial performance of four preservative systems in an oil-in-water cosmetic formulation. In addition to the five standard reference strains, seven environmental and raw-material-associated microorganisms associated with walnut and apricot kernel shells were included. All preservative-containing formulations fulfilled the required acceptance criteria; however, marked differences in time-dependent antimicrobial activity were observed between preservative systems. Among the tested systems, the formulation containing 0.4% potassium sorbate demonstrated the most rapid and broad-spectrum antimicrobial performance across the expanded microbial panel. In contrast, combinations of potassium sorbate with sodium benzoate and benzyl alcohol showed slower antimicrobial efficacy. In conclusion, this study demonstrates that reliance solely on standard reference microorganisms may underestimate preservation risks in cosmetics that use naturally derived raw materials. The adaptive preservation challenge test is a risk assessment approach for evaluating the microbiological stability of cosmetic formulations with reduced-preservative systems.

Keywords: cosmetics; microbial contamination; adaptive preservation test; risk-based approach



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1. Introduction

Natural cosmetic products are susceptible to microbial contamination during production and use may compromise their safety and quality [1]. In a survey of 100 unused cosmetic products from the United Arab Emirates market, microbial contamination was tested. Thirteen percent of the samples were contaminated with yeasts and molds, while 15% exceeded the maximum acceptable limits for aerobic mesophilic bacteria, indicating inadequate microbiological quality [2]. In a similar investigation conducted in Italy of used cosmetic products, including creams, lotions, masks, serums, and hair care products, approximately 20–30% of the samples were contaminated with potentially harmful microorganisms. In particular, an increase in Gram-negative bacteria was observed during product use over time, which was attributed to repeated consumer handling and a progressive decline in preservative efficacy [3]. To prevent microbial growth in products,

preservative systems are added regularly and can consist of single preservatives or combinations of multiple agents that work together. Commonly used chemical preservatives include parabens, phenoxyethanol, organic acids, and formaldehyde-releasing agents [4]. Microbial contaminants may originate from susceptible raw materials, including water, as well as from production environments or consumer handling. They may cause changes in the product's color, texture, fragrance, and effectiveness and may pose health risks, especially when applied to sensitive or damaged skin [5]. Consequently, the antimicrobial protection of cosmetics must be evaluated using standard preservation challenge tests prior to market release. Several international guidelines and standards are available for evaluating preservation efficacy, including the USP 51 antimicrobial effectiveness test in the United States [6], the ASEAN Cosmetic Guidelines in Southeast Asia [6], the PCPC methods in the United States [4], and the SCCS guidance in the European Union [7]. In Europe and many other regions, EN ISO 11930 is the standard method for assessing the efficacy of cosmetic preservative systems [8]. Interestingly, these tests were originally designed to assess the preservation of pharmaceutical products using a standard range of Gram-negative and Gram-positive bacteria, yeast, and mold [9]. These test strains are deliberately chosen to represent a broad range of microorganisms, usually comprising a "worst case" scenario. However, there is virtually no evidence regarding the efficacy of preservative systems on real contaminants. Consequently, product manufacturers may face failure of their preserved formulas due to the occurrence of specific microbial contaminants. Thus, EN ISO 11930, despite its almost exclusive use in Europe, might not address microbial risks associated with production facilities or contaminations originating from natural raw materials. In contrast, the SCCS guidelines follow a product-specific risk-based approach, and some alternative methods include test strains that may degrade cosmetic products in the challenge test [7,10]. The standard methods, such as USP 51 and EN ISO 11930, explicitly acknowledge the importance of a risk-based approach in assessing preservation efficacy; however, both continue to rely on a limited set of predefined reference microorganisms for routine antimicrobial effectiveness testing, which may not fully represent emerging or product-specific contaminants [6,8]. Although many manufacturers may be aware of this problem and thus adapt their own challenge test protocols, there is very little published scientific information on the efficacy of preservation systems against non-standard microorganisms, including those used in the Schülke KoKo test [10]. Especially for cosmetic products containing minimally processed natural raw materials, this issue may pose a challenge. Pretreatment of these raw materials may be difficult and may cause additional costs, which might prevent raw material suppliers from low- and middle-income countries from accessing the European market. In our study, we focused on walnut shells (WS) and apricot kernel shells (AKS) of Central Asian origin, which can be used as natural exfoliating agents. Metagenome analyses of these materials revealed complex contamination with several plant-associated and environmental microorganisms, including potential opportunistic pathogens [11]. Most of the identified microorganisms are not covered by EN ISO 11930:2019 but may persist in cosmetic formulations and may pose a challenge to preservative systems under real-use conditions.

Therefore, we aimed to evaluate the antimicrobial efficacy of four preservatives added to a common cosmetic formulation using a preservation test approach in accordance with EN ISO 11930. In addition to the standard test strains, selected microorganisms identified to be present in WS and AKS (i.e., *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Fusarium oxysporum*, *Enterobacter hormaechei*, *Ralstonia pikei*, *Erwinia billingiae*, and *Enterobacter cloacae*) were included. With this approach, the study aims to assess the performance of preservatives and improve microbiological risk evaluation for modern natural cosmetic formulations. In this regard, different preservative systems used for this study were chosen

from different chemical groups (i.e., organic acids, pentylene glycol and benzyl alcohol) and also considering mixtures, which are frequently used in cosmetic formulations. Thus, the data might help extrapolate putative risks associated with microbial contaminants not covered by standardized methods, thus enabling the prudent and predictive adaptation of common preservation systems.

2. Materials and Methods

2.1. Cosmetic Formulation and Sample Preparation

A basic oil-in-water (O/W) emulsion was prepared as an unpreserved control formulation (base formulation) using the ingredients listed in Table 1. Four preservative-containing formulations were prepared by adding different preservative systems to the same base formulation (Table 2). The pH of each was measured as mentioned in Table 2 prior to testing against selected microorganisms in the preservation challenge test. To prepare the O/W emulsion, the aqueous phase (water, glycerin, xanthan gum) and the oil phase (caprylic/capric triglyceride, glyceryl stearate, cetearyl olivate/sorbitan olivate, tocopherol blend, and cetearyl alcohol) were prepared separately. Both phases were heated to approximately 80 °C, and homogenized using an IKA Magic Lab (IKA-Werke GmbH & Co. KG, Staufen, Deutschland) under continuous stirring to form an emulsion. The mixture was homogenized until uniform, then cooled to below 45 °C while stirring. Preservatives were added to the cooled emulsion at temperatures below 40 °C. All formulations were prepared under hygienic conditions using sterile tools and containers. The pH of each formulation was measured at 25 °C using a calibrated pH meter (VWR International GmbH, Darmstadt, Deutschland) and is presented in Table 2. Formulations were stored at 2–4 °C in a refrigerator until use.

Table 1. Basic oil-in-water formulation.

Ingredients According to INCI	Pure Substance Content (%)	Raw Material Preparation (%)
Aqua	100	Ad 100
Glycerin (Mercur Handel GmbH, Hamburg, Germany)	85	5.00
Caprylic/Capric Triglyceride Glyceryl Stearate (Wilmar Europe Trading B.V., Rotterdam, Netherlands)	100	15.00
Glyceryl Stearate BASF SE, Ludwigshafen am Rhein, Germany)	100	2.50
Cetearyl Alcohol (Ecogreen Oleochemicals GmbH, Dessau-Roßlau, Germany)	100	2.00
Cetearyl Olivate, Sorbitan Olivate (C.H. Erbslöh GmbH & Co. KG, Krefeld, Germany)	100	2.00
Tocopherol, Helianthus Annuus seed oil (Novoclon GmbH, Pforzheim, Germany)	100	0.80
Xanthan Gum (BASF SE, Ludwigshafen am Rhein, Germany)	100	0.25
Lactic acid (Jungbunzlauer Austria AG, Wien, Austria)	100	1.00

2.2. Microorganisms

Preservative performance was tested against both the standard EN ISO 11930 reference organisms and additional environmental and raw-material-associated microorganisms as-

sociated with walnut shells (WS) and apricot kernel shells (AKS) [11]. ISO reference strains included *Escherichia coli* (American Type Culture Collection (ATCC) 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404) [8]. Additionally, *Pantoea agglomerans* (Deutsche Sammlung von Mikroorganismen (DSMZ) 3493), *Enterobacter cloacae* (DSMZ 26481), *Enterobacter hormaechei* (DSMZ 12409), *Ralstonia pickettii* (DSMZ 6297), *Erwinia billingiae* (DSMZ 17872), *Fusarium oxysporum* (DSMZ 62306), and *Pseudomonas aeruginosa* (DSMZ 50071) were included to enable comparison between standard and non-standard strains relevant to raw-material contamination risks [8]. Microbiological experiments with opportunistic pathogens such as *P. aeruginosa* and *F. oxysporum* were handled under Biosafety Level 2 (BSL-2) laboratory conditions in accordance with institutional biosafety guidelines.

Table 2. Formulations using the basic formula.

Formulation	Preservative(s)	pH
C (Control)	None	5.2
F1	5% Pentylene glycol (Cosphatec GmbH, Hamburg, Germany)	5.9
F2	0.4% Potassium sorbate (Nantong Acetic Acid Chemical Co., Ltd., Nantong, Jiangsu, China)	5.3
F3	0.25% Potassium sorbate + 0.25% Sodium benzoate (Gansu Kinbo Industry Co., Ltd., Lanzhou, Gansu, China)	5.3
F4	1% Benzyl alcohol (Düllberg Konzentra GmbH & Co. KG, Hamburg, Germany)	5.5

2.3. Culture Media and Preparation of Microbial Inoculum

Tryptic Soy Agar (TSA, Thermo Scientific Oxoid, Wesel, Germany) was used to cultivate bacterial strains, which were incubated at 30–37 °C for 24–48 h. *Enterobacter cloacae* was cultivated exclusively on Trypticase Soy Yeast Extract Agar (TSYEA) and incubated at 37 °C for 48 h. TSYEA was prepared in accordance with DSMZ Medium 92. The medium components were dissolved in appropriate containers and sterilized by autoclaving at 121 °C for 15 min, and the pH was adjusted to 7.0–7.2 after sterilization [12]. *Candida albicans* was cultured on Sabouraud Dextrose Agar (SDA; Thermo Scientific Oxoid, Wesel, Germany) at 30 °C for 48 h. *Fusarium oxysporum* was grown on Potato Dextrose Agar (PDA; Thermo Scientific Oxoid, Wesel, Germany) at 30 °C for 4–5 days, while *Aspergillus brasiliensis* was cultivated on Malt Extract Agar (MEA; Thermo Scientific Oxoid, Wesel, Germany) under the same incubation conditions. Stock and working cultures were prepared in accordance with EN ISO 11930 guidelines and stored at 2–4 °C until use. Microbial inocula were standardized to concentrations of 1×10^7 – 10^8 CFU/mL for bacterial strains and 1×10^6 – 10^7 CFU/mL for yeasts and molds. Inoculum concentrations were calibrated spectrophotometrically using a GENESYS spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.4. Neutralizer Preparation

The standard neutralizer specified in EN ISO 11930 was tested against the selected microorganisms for each formulation and found unsuitable for all formulations. Therefore, a new neutralizer was prepared using the ingredients listed in Table 3 and sterilized by autoclaving at 121 °C for 15 min. To maintain uniform experimental conditions, the standard microbial strains were also tested with the same neutralizer.

Table 3. Modified neutralizer.

Ingredient	Amount
Polysorbate 80	30 g·L ⁻¹
Lecithin	3 g·L ⁻¹
L-cysteine	1 g·L ⁻¹
Distilled water	to 1 L

2.5. Neutralizer Suitability Test

Neutralizer suitability was assessed independently for each microorganism and each formulation, and the recovery rate results are provided in Supplementary File S1. For each test, three tubes were prepared, whose measurements are as follows: inoculum tube (1 mL of bacterial suspension + 10 mL of diluent), a test tube (1 g of formulation + 9 mL of neutralizer + 1 mL of bacterial suspension), and a control tube (9 mL of neutralizer + 1 mL of bacterial suspension + diluent in place of formulation). Following mixing, tenfold serial dilutions were prepared to 10⁻³ or 10⁻⁴, and 100 µL of each dilution was spread-plated onto the respective agar plates using the spread plate method. Plates were incubated under organism-specific conditions.

2.6. Adaptive EN ISO 11930 Preservation Challenge Test Protocol

A preservation challenge test was performed using a reference method from EN ISO 11930 with adaptations to include additional microorganisms and modified sampling intervals. Ten grams of formulation were inoculated with 100 µL of the prepared suspension. The bacterial suspension was adjusted to a concentration of 1 × 10⁷–1 × 10⁸ colony-forming units (CFU) per mL, and the yeast and mold suspension was adjusted to 1 × 10⁶–1 × 10⁷ CFU/mL. The initial microbial plate counts for each inoculum are included in Supplementary File S2. After inoculation, the formulations were stored at 25 °C throughout the study. One gram of inoculated formulation was transferred into 9 mL of modified neutralizer (Table 3) and mixed thoroughly at each sampling time (initial count (0 h), 3 h, Day 1, Day 7, Day 14, and Day 28). To ensure effective neutralization of the preservative system, the mixture was kept at room temperature for 30 min. Serial dilutions were prepared from each neutralized sample, plated using the spread plate method, and incubated at organism-specific temperatures. Microbial counts were determined to assess preservative effectiveness over time [8].

2.7. Data Analysis and Visualization

After incubation, plates containing 30–330 colonies were set at the microbial enumeration limit of detection. Colony counts were expressed as CFU/mL and log₁₀ transformed prior to analysis to normalize microbial count distributions. Log₁₀ microbial reductions were calculated relative to the initial count (0 h) for each microorganism and formulation at each sampling time point using the equation

$$\text{Log reduction} = \log_{10}(N_0) - \log_{10}(N_t)$$

where N₀ represents the initial microbial counts (0 h), and N_t represents the microbial counts at the respective sampling time point.

For statistical soundness, each experiment was performed in three independent replicates. Results are presented as mean log₁₀ (CFU/mL) ± standard deviation (SD). A heatmap of logarithmic reduction over time across all the formulations was created using GraphPad Prism version 10.6.1. The data was analyzed using RStudio (version 4.4.3) with the ggplot2 package for line graphs. It represents time-dependent microbial reduction, with mean

$\log_{10}(\text{CFU/mL})$ values plotted for each time point (0 h, 3 h, Day 1, Day 7, Day 14, and Day 28) (Supplementary File S3, mean $\log_{10}$ values over time using the ISO criterion). In detail, log reductions across the formulations relative to the base formulation, with statistical significance (p -values), were analyzed using two-way ANOVA with Dunnett's multiple comparisons test (Supplementary File S4).

3. Results and Discussion

The antimicrobial performance of various preservation systems was evaluated against the control base formulation over 28 days. Microbial counts in CFU/mL were monitored at the initial time point, 3 h, Day 1, Day 7, Day 14, and Day 28, as shown in Figure 1.

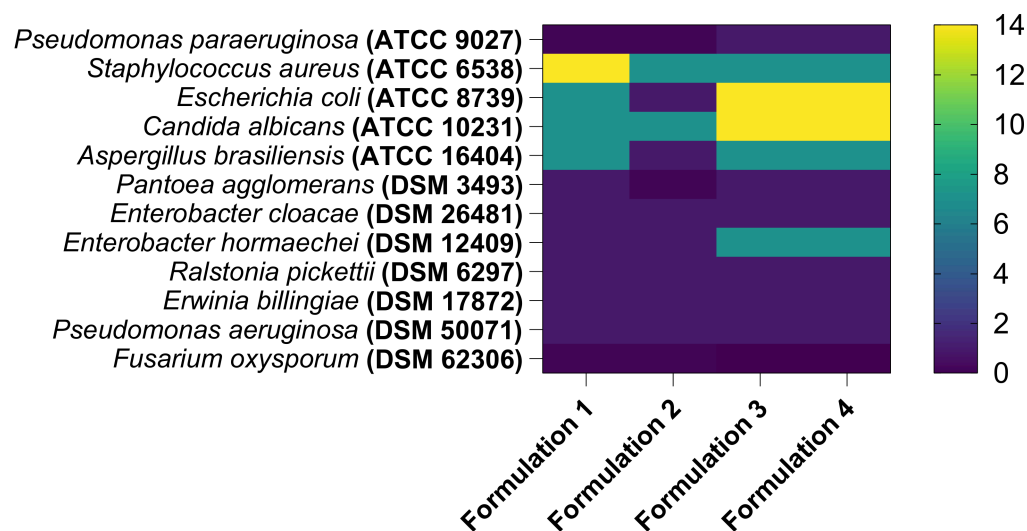


Figure 1. Logarithmic reduction time comparison in days across all organism-formulations to pass the ISO 11930 challenge test criterion.

The results demonstrate a time-dependent reduction in microbial counts across all formulations, with efficacy varying by formulation composition (Figures 2–6). According to the EN ISO 11930 protocol, the control formulation without preservative did not meet criterion A (Table 4) for any microorganism. In contrast, formulations containing preservatives fulfilled Criterion A, as shown in Table 4.

Table 4. Logarithmic reduction for each microbial strain to pass the preservation challenge test based on the ISO11930 criterion [8].

Microorganism	Formulation 1	Formulation 2	Formulation 3	Formulation 4	ISO Pass Criterion *
<i>P. paraeruginosa</i>	6.49	6.49	6.75	6.75	≥ 3 nd NI (From day 7)
<i>S. aureus</i>	7.52	7.49	3.35	4.94	
<i>E. coli</i>	5.59	5.47	5.86	5.99	
<i>C. albicans</i>	1.15	3.11	3.04	2.59	≥ 1 and NI (From day 7)
<i>A. brasiliensis</i>	5.56	5.04	5.77	6.13	≥ 1 and NI (From day 28)
<i>P. agglomerans</i>	4.73	3.92	4.60	4.46	≥ 3 and NI (From day 7)
<i>E. cloacae</i>	4.63	4.71	4.62	4.63	
<i>E. hormaechei</i>	4.75	4.81	4.69	4.70	
<i>R. pickettii</i>	5.50	5.61	5.69	5.50	
<i>E. billingiae</i>	4.07	4.14	4.12	4.13	
<i>P. aeruginosa</i>	4.99	5.02	5.11	5.02	
<i>F. oxysporum</i>	2.52	2.48	7.00	7.00	≥ 1 and NI (From day 28)

* The deviation of 0.5 log is acceptable. * NI = no increase in count since the last sampling. * Rx = 0 if $\lg N_0 = \lg N_x$ (no increase since the first count).

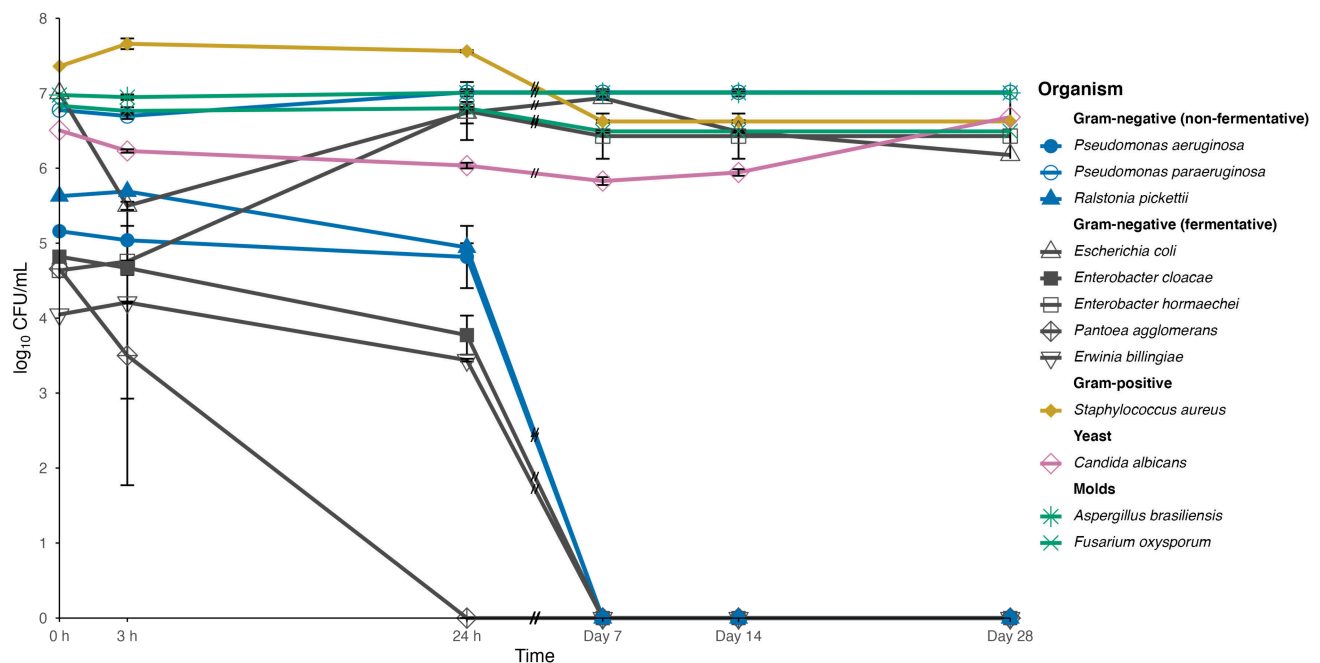


Figure 2. Microbial counts in control formulation (no preservative added) at pH 5.2.

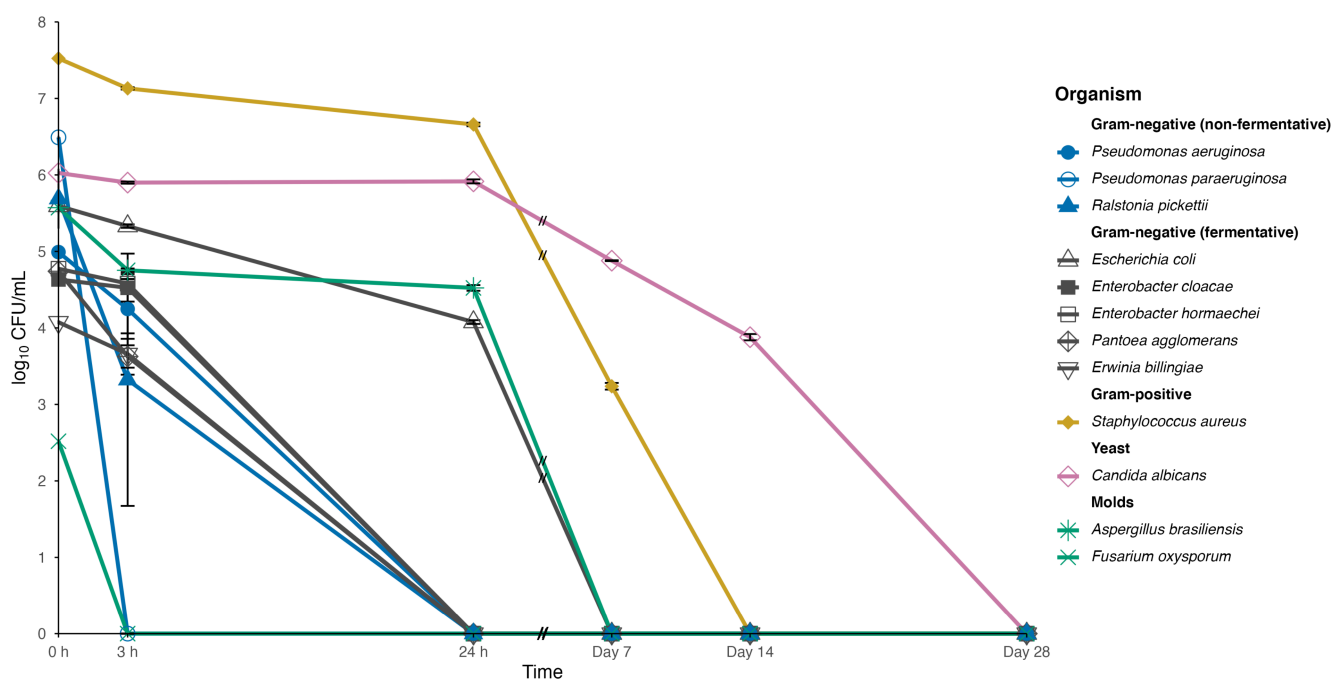


Figure 3. Microbial counts in formulation 1 with 5% pentylene glycol preservative at pH 5.9.

Natural formulations have minimal adverse effects but may be easily compromised by microbial contamination [1,13]. Although all formulations passed the regular quality check, natural, sustainable, and low-preservative-containing cosmetics may be susceptible to microbial spoilage and contamination during consumer use, potentially compromising product safety. A three-year study of the RAPEX database reported *Pseudomonas aeruginosa* as the most frequently detected pathogen in microbiologically contaminated cosmetics recalled from the EU market [14]. In 2025, “EU Safety Gate cosmetics contamination alerts” also included multiple microbiologically contaminated cosmetic products, including children’s products such as sunscreens and face paints. *Pseudomonas aeruginosa* and other opportunistic contaminants such as *Burkholderia cepacia*, *Serratia marcescens*, *Enterobacter*

spp., yeast, *Staphylococcus aureus*, *Gluconacetobacter liquefaciens*, and *Paenibacillus glucanolyticus* were detected in recalled products [15]. The recurrence of microbial contamination in products that have undergone routine quality control highlights the need to update standard quality-control protocols to include risk-associated microorganisms.

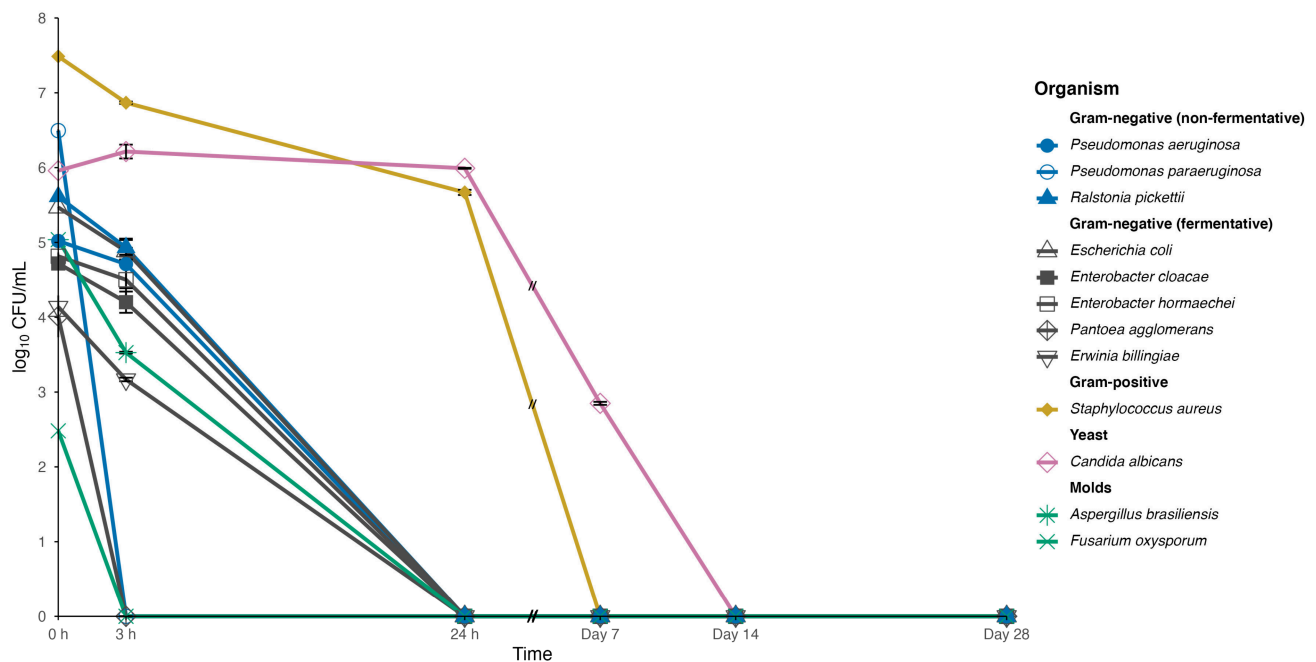


Figure 4. Microbial counts in formulation 2 with 0.4% potassium sorbate preservative at pH 5.3.

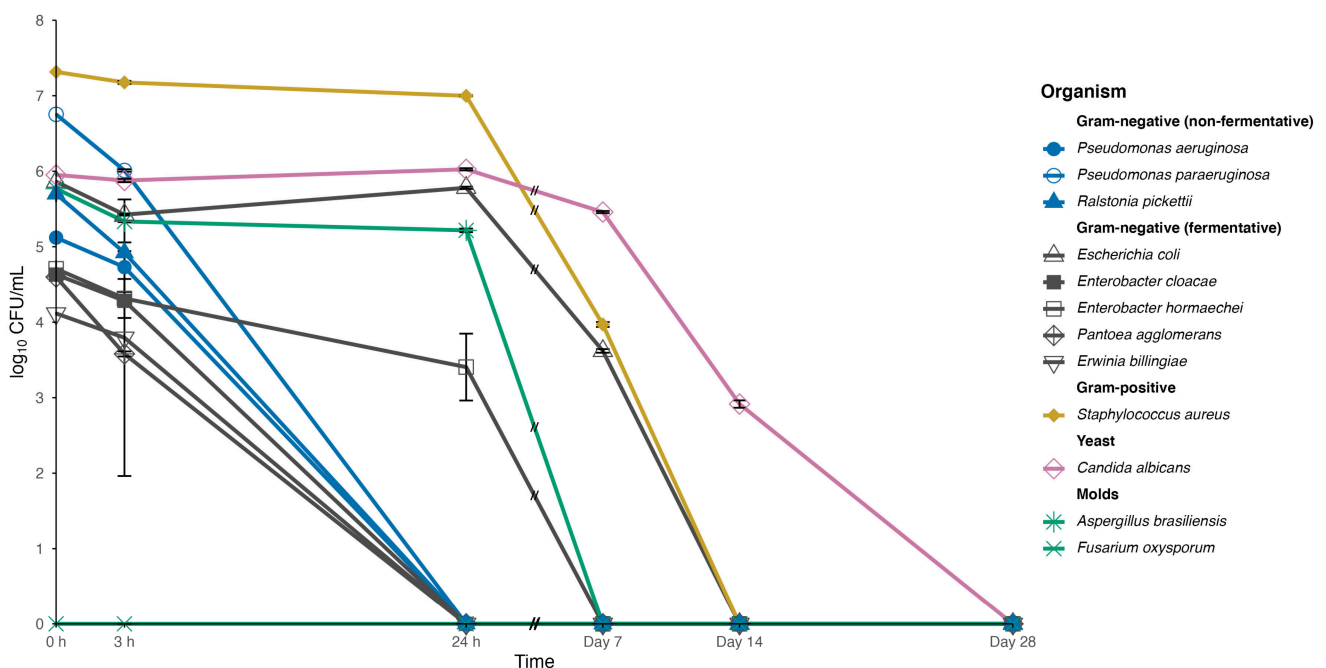


Figure 5. Microbial counts in formulation 3 with 0.25% potassium sorbate + 0.25% sodium benzoate preservative at pH 5.3.

Figure 2 (control base formulation) shows constant or slight variations in microbial counts throughout the observation period. Complete microbial inhibition was not achieved for any tested organism due to the absence of a preservative substance in the formulation.

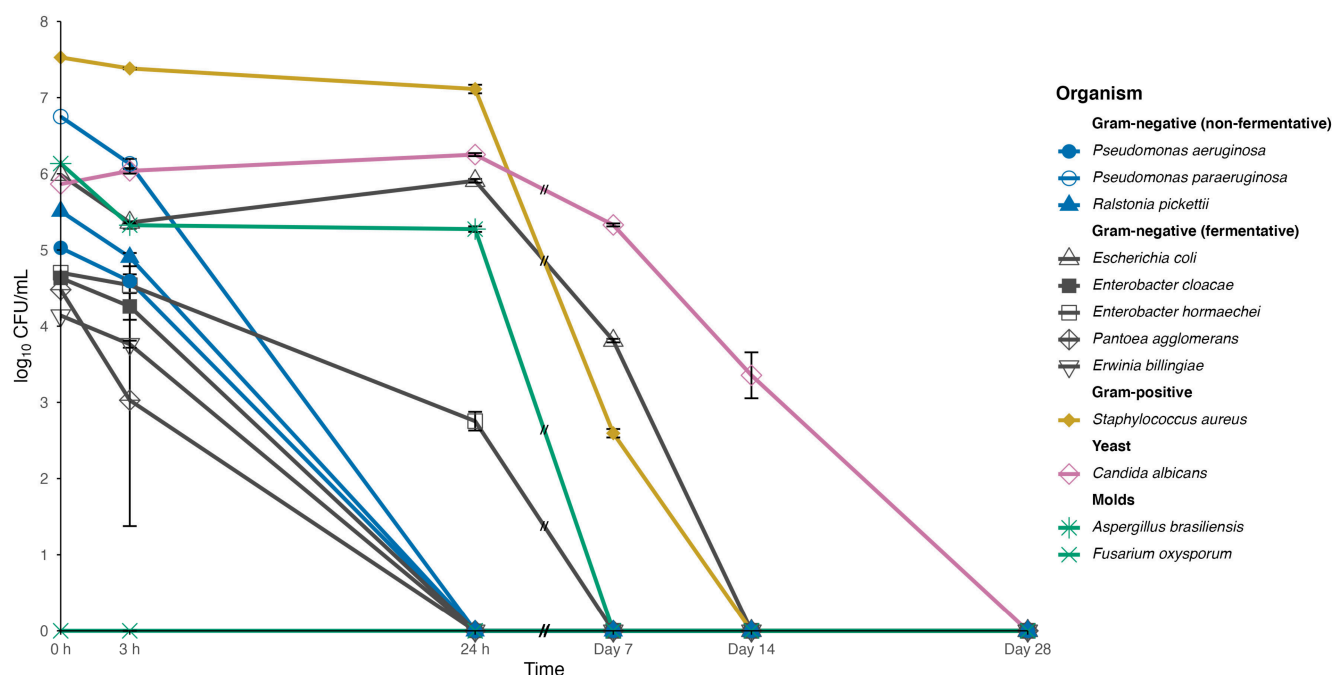


Figure 6. Microbial counts in formulation 4 with 1% benzyl alcohol preservative at pH 5.5.

As illustrated in Figure 3, a greater reduction in microbial counts was observed in formulation 1 compared to the base formulation. Gram-negative fermentative bacterial strains reached non-detectable levels within 24 h, except for the standard strains *E. coli* and *S. aureus*. Yeast showed a gradual decline and remained detectable until Day 28. Formulation 2 exhibited the highest antimicrobial efficacy; even *Candida albicans* achieved a marked reduction by Day 14. The present study evaluated the efficacy of various preservative systems against an expanded range of microorganisms relevant to raw materials used in natural cosmetics. In Figure 4, formulation 2 containing 0.4% potassium sorbate achieved the earliest kill efficiency across a wide spectrum of microorganisms within 14 days, whereas formulation 3 containing 0.25% potassium sorbate + 0.25% sodium benzoate required up to 28 days to reach comparable reduction at similar pH (Figure 5). This indicates that preservative combinations at lower individual concentrations may lead to delayed antimicrobial responses compared with a single preservative system under comparable physicochemical conditions. It supports earlier studies showing that preservative formulations may pass regulatory challenge tests yet fail under real-use conditions [16].

Preservatives such as potassium sorbate and sodium benzoate are commonly used in mildly preserved “natural” cosmetic products; however, their antimicrobial efficacy decreases near neutral pH [17]. Consistent with this, previous studies have shown that weak organic acids are less effective against Gram-negative bacteria, particularly at near-neutral pH conditions [18].

In contrast, our study showed the early killing of Gram-negative fermentative and non-fermentative bacteria with the 0.4% potassium sorbate preservative at pH 5.27 (Figure 4). Therefore, this may indicate that even minor pH shifts in formulations with low-preservation systems can increase susceptibility to contamination after-market release, potentially contributing to product failures observed under real-use conditions.

As shown in Figure 6, formulation 4 exhibited a gradual reduction in microbial activity, primarily for the ISO 11930 reference strains. However, *P. agglomerans* achieved the pass criterion within 3 h. In contrast to weak organic acids, preservatives such as benzyl alcohol

and pentylene glycol exhibit limited pH dependence and offer broader antimicrobial efficacy [19,20]. Pentylene glycol (5%) is pH-independent and showed an overall moderate lethal efficiency (73.9%) but required more time to act against Gram-positive bacteria and yeast (Figure 3). Cosmetic formulations containing natural raw materials, therefore, require enhanced preservative systems to achieve broad-spectrum antimicrobial effectiveness. By incorporating additional environmental and raw-material-associated microorganisms, evaluating a low-preservation system against risk-based organisms, validating neutralization procedures, and adapting sampling intervals, the present study proposes an adaptive preservation challenge test approach.

ATCC 9027 belongs to the PA7-related outlier lineage of the *P. aeruginosa* complex, which has been proposed as a separate species, *Pseudomonas paraaeruginosa*, and is widely used for antimicrobial and biofilm studies [21]. The inclusion of the type strain DSM 50071, belonging to the sensu stricto lineage, which comprises the majority of *P. aeruginosa* strains, allowed comparison with a standard representative of the species [22]. No significant difference between the two species.

Adaptive and risk-based preservative efficacy testing approaches have previously been discussed in SCCS recommendations and implemented in concepts such as the Schülke KoKo test, which includes organisms associated with product degradation [7,10]. The present study provides a more systematic comparison of preservative kinetics across an expanded panel, particularly for preservative-reduced cosmetic formulations containing Central Asian botanical abrasives.

Taken together, the findings of this study support the inclusion of environmental and raw-material-associated microorganisms, particularly for high-risk cosmetic formulations. Our study evaluated preservative efficacy exclusively in a single oil-in-water (O/W) formulation; future research may focus on other cosmetic formats, such as anhydrous, W/O, and surfactant-rich systems with improved preservatives. However, formulation-specific characteristics such as pH, ingredient interactions, and matrix composition are known to influence preservative performance; the current experimental design was not intended to quantify the contribution of these individual factors. Degradation analysis of preservative concentration was ruled out for late-stage microbial recovery at day 28. Consumer-use simulations, such as repeated opening, finger-dipping, or temperature cycling, to better test preservation challenges. Real-world contamination often involves polymicrobial communities; a pilot experiment using a defined mixed inoculum could be conducted to provide more realistic conditions and improve microbiological risk assessment of modern cosmetic products.

4. Conclusions

This study demonstrates that standard preservation challenge tests, such as USP 51, ASEAN guidelines, PCPC methods, SCCS recommendations, and EN ISO 11930, may not alone fully capture the microbiological risks associated with cosmetic formulations containing Central Asian botanical abrasives. By including environmental and raw-material-associated microorganisms, the adaptive EN ISO 11930 preservation challenge test revealed that preservative efficacy depends primarily on the type of preservative used in formulation. Among the tested preservation systems, formulation 2, containing 0.4% potassium sorbate, consistently exhibited broad-spectrum antimicrobial activity in the oil-in-water (O/W) formulation. The single-preservative system, such as 0.4% potassium sorbate, could be more efficient than the combined preservation system containing 0.25% potassium sorbate and 0.25% sodium benzoate in achieving a faster antimicrobial response. On the other hand, 5% pentylene glycol is pH-independent and multifunctional, unlike 0.4% potassium sorbate, but it showed moderate antimicrobial efficacy with slower re-

duction, particularly against Gram-positive bacteria and yeast. Although all preserved formulations met ISO 11930 Criterion A, the inclusion of risk-associated environmental microorganisms revealed meaningful differences in early-, moderate-, and late-reduction kinetics that may be critical under real-use conditions. Overall, our data suggest a supplementary formulation-specific extended microbial quality-check approach for natural cosmetics with reduced-preservative systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cosmetics13040168/s1>. The Supplementary File contains neutralizer suitability for each microorganism in File S1 and the initial inoculum count in File S2. Mean log₁₀ values over time using the ISO criterion added in File S3, and log reductions across the formulations relative to the base formulation included in File S4. The data presented in this study are available in the Supplementary Materials at Zenodo <https://doi.org/10.5281/zenodo.20752841> (accessed on 18 June 2026).

Author Contributions: Conceptualization, D.P.B. and B.D.; methodology, B.D. and G.F.; software, B.D.; validation, D.P.B.; formal analysis, B.D. and G.F.; investigation, B.D.; resources, D.P.B., and R.B.; data curation, D.P.B.; writing—original draft preparation, B.D., M.E.D. and D.P.B.; writing—review and editing, B.D., M.E.D., R.B. and D.P.B.; visualization, D.P.B. and B.D.; supervision, D.P.B.; project administration, D.P.B.; funding acquisition, D.P.B. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

AKS	Apricot Kernel Shells
ASEAN	Association of Southeast Asian Nations
ATCC	American Type Culture Collection
CFU	Colony Forming Units
DSM	Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms and Cell Cultures)
EN	European Norm
EU	European Union
IKA	IKA Laboratory Equipment
ISO	International Organization for Standardization
MEA	Malt Extract Agar
NI	No Increase
O/W	Oil in Water
PDA	Potato Dextrose Agar

PCPC	Personal Care Products Council
RAPEX	Rapid Alert System for Dangerous Non-Food Products
SCCS	Scientific Committee on Consumer Safety
SDA	Sabouraud Dextrose Agar
TSA	Tryptic Soy Agar
TSYEA	Trypticase Soy Yeast Extract Agar
USP	United States Pharmacopeia
WS	Walnut Shells

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