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Modification of Superabsorbent Polymer Granules and Fibers for Antimicrobial Efficacy and Malodor Control

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Cite This: *ACS Omega* 2024, 9, 10201−10206

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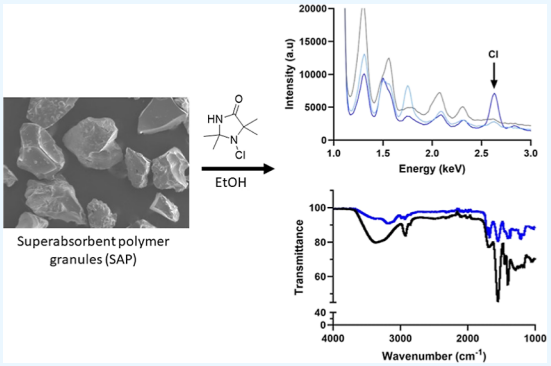
ABSTRACT: Superabsorbent polymer (SAP) granules, typically used in personal care devices such as diapers, incontinence devices, hygiene pads, and wound dressings, and granular particles of zeolite and bentonite were each subjected to modification by exposure to solutions of 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC) in ethanol at room temperature. The air-dried granules showed newly acquired properties attributable to the presence of active chlorine (Cl^+). The treated particles effectively oxidized the malodorant 3-mercapto-3-methylbutanol (3M3MB). MC-treated granules inactivated urease, a microbial exoenzyme commonly involved in ammonia production. Modified SAP granules and superabsorbent fibers (SAFs) showed powerful antibacterial activity in an in vitro chronic wound model. The results suggest that processing of SAP granules and SAFs by this simple method at an industrial scale could add value to their widespread use in a variety of personal hygiene devices and specifically to the improvement of chronic wound care.



INTRODUCTION

An estimated three million tons of superabsorbent polymer (SAP) granules are produced annually, primarily for use in absorptive cores of diapers, incontinence devices, mattress pads, and feminine hygiene pads.[1,2] SAPs are also incorporated into wound dressings to improve wound healing.[3−5] Retention of biological fluids with these devices is accomplished by the massive absorptive capacity of the cross-linked polymer granules, ranging between 10 and 1000 g of water per gram of SAP. These are often combined with high-surface-area cellulose fluff pulp fibers, which rapidly disperse liquid but have limited absorptive capacity.[2,6] In many applications, the accumulation of organic materials in these products results in microbial growth, exoenzyme secretion, and the formation of malodorous volatile reaction products.[7] Emanation of odors from these devices during use becomes a source of inconvenience and embarrassment; malodor release in the waste disposal stream is also problematic.[8−10]

Mitigation of malodor formation by modification of SAP granules is made difficult by the swelling that occurs in aqueous conditions, requiring that a modification process be compatible with nonaqueous solvents. To the best of our knowledge, there are currently no industrial-scale methods to modify SAP granules for effective odor control. Here, we describe SAP granule treatment through a brief soaking procedure in ethanol containing 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC), a compound that confers upon the air-dried particulates stable oxidizing and halogenating properties (Figure 1).[11] We show that this treatment confers odor control through three possible pathways: (A) antimicrobial activity in a chronic wound model; (B) inhibition of urease, a microbial exoenzyme that is responsible for the formation of ammonia; and (C) modification of malodorous volatile small molecules. These became possible without altering the absorptive capacity of the granules.

METHODS

Reagents. *Canavalia ensiformis* (Jack Bean) urease, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), and the ammonia assay kit (AA0100) were purchased from Sigma-Aldrich. 3-Mercapto-3-methylbutanol (3M3MB) was purchased from Biosynth Carbosynth. Urea, sodium thiosulfate, 2-(4-morpholino)ethanesulfonic acid (MES), and absolute ethanol (18-602-522) were purchased from Fisher Scientific. 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC), zeolite, and bentonite were provided by MedeSol LLC, Bellevue, WA. SAP granules were purchased from Science Gone Fun. Free available chlorine reagents were purchased from Hach Company (Loveland, CO). Water from Milli-Q water

Received: September 20, 2023
Revised: February 2, 2024
Accepted: February 8, 2024
Published: February 22, 2024

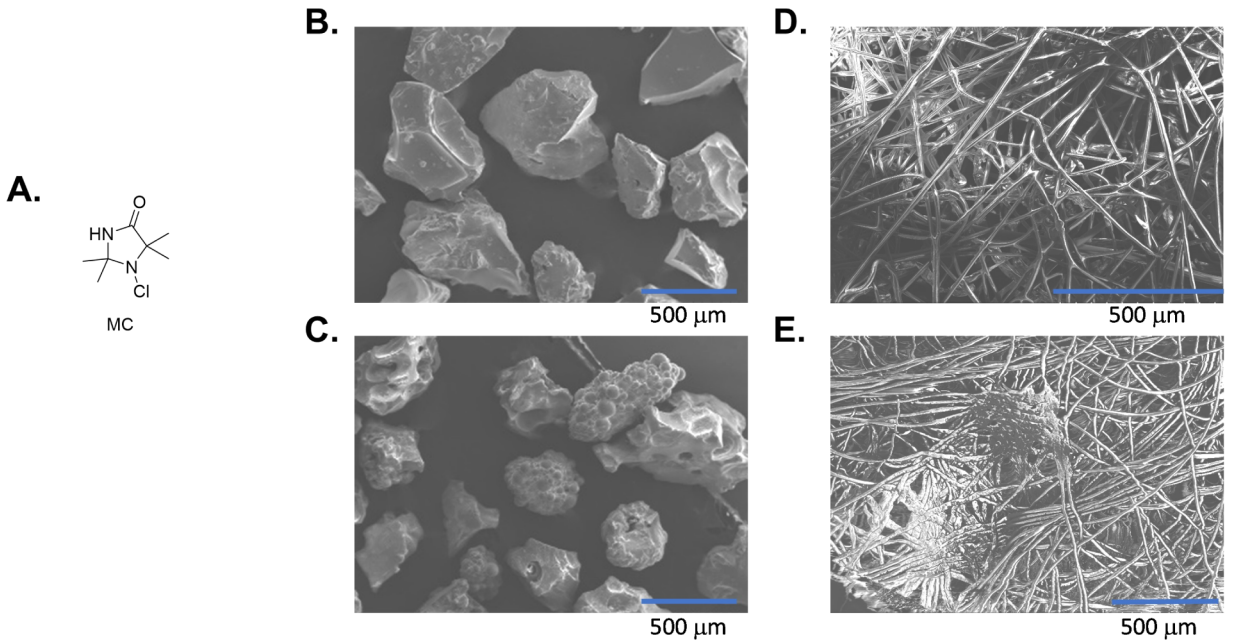


Figure 1. Structure of MC and SEM images of SAP granules and SAFs. (A) Structure of MC. (B) SEM image of SAP granules before the addition of MC where only plate-like granules were detected. (C) SEM image after treatment with MC showing plate-like and spherical granules. (D) SEM image of SAF no. 2351. (E) SEM image of SAF no. 2344.

purification system (MilliporeSigma, Burlington, MA) was used for all experiments.

Preparation of MC-Treated SAP, Bentonite and Zeolite Granules, and SAFs. A 5% (m/v) MC solution was freshly prepared in absolute ethanol. Samples were prepared by adding 500 mg of SAP granules to 10 mL of 5% MC solution. The samples were incubated for 30 min. The solution was swirled by hand periodically throughout the incubation period. The solution was decanted, and the granules were air-dried at ambient temperature for a minimum of 1 h to allow any remaining ethanol to evaporate. Solids were then ready for use or stored in the dark at room temperature before being used later. MC-treated bentonite and zeolite samples were prepared by following the same procedure. All control granules were incubated with absolute ethanol for 30 min and air-dried as described above. The super absorbent fibers (SAFs), product nos. 2344 and 2351, were obtained from Technical Absorbents, Energy Park Way, Grimsby, North East Lincolnshire, DN31 2TT United Kingdom. Following a similar method as described above, a single sheet was submerged in 5% (m/v) MC for 30 min. The SAFs were dried at ambient temperature and stored in the dark at room temperature.

Determination of Free Available Chlorine on MC-Treated Granules and SAFs. MC-treated granules (50 mg) were vortexed in 25 mL of ultrapure water for one min and decanted into a 125 mL Erlenmeyer flask. This step was repeated to yield a final volume of 50 mL. Iodometric titrations using sodium thiosulfate (0.113 N) were completed following the HACH method 8209. A 1 cm^2 piece of SAF was submerged in 50 mL of ultrapure water. After 5 min, the titration was completed as described above. Untreated SAP granules and SAFs were titrated for residual chlorine following the same procedure.

Absorptive Capacity of MC-Treated SAPs. The total absorptive capacity of MC-treated and untreated SAP granules was measured following a published procedure.[2,12] 50 mg of SAP granules were placed inside a tea filter. The weight of the empty dry tea filter with the SAP granules was recorded (W_1). The tea filter containing the granules was fully submerged in a 600 mL beaker filled completely with ultrapure water for 30 min. The tea filter was removed and hung to remove excess water for 30 min. The tea filter was lightly shaken twice to remove any excess water, and the weight was recorded (W_2). The total absorptive capacity of the SAP granules was calculated by the following equation: absorptive capacity (%) $= \frac{W_2}{W_1} \times 100$ where W_2 is the weight of the water-treated tea filter containing SAP granules, and W_1 is the weight of dry tea bag and SAP granules.

Quantitation of 3M3MB Modification with MC-Treated Granules. Aqueous trials were evaluated using 10 mg of MC-treated granules in 3.5 mL of 1 mM 3M3MB in 50 mM MES buffer (pH 6.8). Samples were incubated for 5 and 30 min, with occasional inversion of the tubes. Samples were centrifuged using a Beckman Coulter microfuge 20R at 5000 rpm for 2 min. 50 μL of the supernatant was added to a cuvette containing 900 μL of 50 mM MES (pH 6.8) and 50 μL of 20 mM DTNB. The solutions were incubated for 5 min. The absorbance of TNB^- was measured at 412 nm using a Thermo Scientific BioMate UV−Vis spectrophotometer. Ethanol trials were evaluated using 200 μL of 1 mM 3M3MB in ethanol. 50 μL of the supernatant was taken directly from the reaction mixture and added to a cuvette containing 900 μL of 50 mM MES and 50 μL of 20 mM DTNB and measured at 412 nm, as previously described.[13] Control samples were processed following the same method.

Determination of Major Products of 3M3MB with MC. Solutions of MC (1 mg/mL in ethanol), 3M3MB (1 mg/mL in water), and MC/3M3MB (0.5 mg/mL in 50% ethanol) were prepared. Each sample (10 μL) was analyzed by liquid chromatography-mass spectrometry (LCMS). An Agilent 1290 Infinity UHPLC system coupled to an Agilent 6230B time-of-flight mass spectrometry (TOF-MS) equipped with a dual

https://doi.org/10.1021/acsomega.3c07164
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Agilent Jet Stream electrospray ionization source was used for compound separation and analysis. The separation was performed with an ZORBAX RRHD Eclipse Plus C18 column (50 mm × 2.1 mm i.d., 95 Å, 1.8 μm) using 15% (v/v) acetonitrile with 0.1% (v/v) formic acid in water at a 400 μL/min flow rate for 15 min and a column temperature of 20 °C.

The TOF-MS instrument was operated in positive ionization mode with the following settings: 325 °C drying gas temperature with an 8 L/min flow rate, 350 °C sheath gas temperature with an 11 L/min flow rate, and 35 psig nebulizer pressure. Voltage parameters were set as follows: 3500 V capillary, 65 V skimmer, 1000 V nozzle, and 175 V fragmentor. The data acquisition was performed in the standard mass range ($\leq m/z$ 3200) with 2 GHz extended dynamic range mode at an acquisition rate of 1.0 spectra/s. A solution with reference masses $[M + H]^+$ at m/z 121.0509 (purine), $[M + H]^+$ at m/z 922.0098 (HP-0921) was continuously infused during data collection for mass correction.

Data was analyzed using Agilent Qualitative Analysis software (v 10.0). Spectral peak data was background subtracted using preinjection data.

Determination of Urease Activity after Contact with MC-Treated Granules. A 1 IU/mL stock solution of *C. ensiformis* urease was prepared in 200 mM phosphate buffer at pH 7.0. MC-treated granules (10 mg) were dispersed in 2.825 mL of 200 mM phosphate buffer pH 7.0 for 2 min prior to enzyme exposure. 175 μL of the urease stock solution (1 IU/mL) was then added, and the mixture incubated for 30 min. The samples were vortexed for 30 s after the addition of urease and again after the reaction was complete. The mixture was centrifuged using a Beckman Coulter microfuge 20R at 5000 rpm for 2 min. Any remaining activity of the MC was quenched by the addition of sodium thiosulfate (1% w/v). 180 μL of the quenched reaction mixture was incubated with urea (15 mM) for 5 min. 100 μL of the sample was tested following the Sigma-Aldrich Ammonia Assay Kit protocol (AA0100).

Antibacterial Efficacy of MC-Treated SAP Granules and SAFs in a Two-Species Biofilm Assay. The antibacterial efficacy was tested following our previously reported procedures.[14] *Staphylococcus aureus* EMRSA-15 and *Pseudomonas aeruginosa* ATCC 9027 were routinely cultured at 37 °C on nutrient agar at a 1.5% concentration (Sigma-Aldrich). *S. aureus* and *P. aeruginosa* used in this study are type strains originally of skin origin. For two-species biofilm preparations, each bacterial culture was equilibrated to an OD of 0.1 (A_{650}), equivalent to CFU = 1×10^8. Setting up of the biofilm flow device followed a previous method with the addition of a 0.22 μm syringe filter placed in the inlet tubing to prevent contamination of the fresh media.[14] Equilibrated bacterial cultures were prepared in a 1:1 ratio by mixing 48 μL of each suspension in a sterile microcentrifuge tube to allow the bacterial species to be added to the device simultaneously. Twelve disks of noble agar at a 1.5% concentration were cut, using an 8 mm biopsy punch, and added to the biofilm device using sterile forceps. One 13 mm, 0.22 μm cellulose filter (Millipore) was placed on top of each agar disc, using sterile forceps, and was inoculated with 20 μL of the bacterial mixture. The device was placed in an incubator at 33 °C and connected to a peristaltic pump, which perfused the system with simulated wound fluid [2.34 mM $CaCl_2{\cdot}2H_2O$, 3.75 mM KCl, 9.9 mM NaCl, 100 mM $NaHCO_3$, 3% (w/v) fetal bovine serum] at a flow rate of 0.322 mL/min.[15] The SAFs (1 cm^2) and SAP granules (10 mg) were applied topically following 5 h incubation to allow for biofilm establishment. At 24 h post-treatment, cellulose filters were collected and individually placed into 15 mL conical tubes containing 1 mL of 0.1% (w/v) sodium thiosulfate and vortexed (2200 rpm, 30 s) to homogenize the biofilm. Serial dilutions were prepared from 10^{-1} to 10^{-12}. Ten microliters of each dilution were pipetted in triplicate onto Baird Parker agar (*S. aureus*; Oxoid) or Cetrimide agar (*P. aeruginosa*; Sigma-Aldrich) and incubated at 37 °C, 24 h for total viable count. At the end of each experiment, the device was sterilized using Gerrard Ampholytic Surface Active Biocide (GASAB) disinfectant at a 1:100 dilution, submerged for 24 h, and then placed in deionized water. The tubing was washed through with GASAB disinfectant before autoclaving.

Scanning Electronic Microscopy and Energy Dispersive Spectrometer. Scanning electron microscopy (SEM) images and the energy dispersive spectrometry (EDS) elementary analysis were performed by a field emission scanning electron microscope (Hitachi SU5000) equipped with an EDS system (Oxford X-MaxN 50).

FTIR for Determination of MC on SAP Granules. SAP granules, MC-treated SAP granules, and MC FTIR data were obtained with a Thermo Nicolet iS50 using a diamond ATR module and recorded with 64 scans at 4 cm^{-1} resolution following literature procedures.[16]

RESULTS

After air drying the modified granules, 50 mg of MC-treated SAP granules in 50 mL of water released 80 ppm of active chlorine. For comparison, zeolite and bentonite granules that underwent the same treatment yielded concentrations of 87 and 71 ppm, respectively. Little change in the concentration of the active chlorine was seen after 3 weeks; after 7 months, the active chlorine concentration was 45 ppm. SEM/EDS was used to confirm the presence of chlorine on both SAP granules and SAP-containing fibers (Figure 1 and Supporting Information). The granules originally showed a plate-like structure. After treatment with MC, plate-like and spherical granules were detected by SEM. Chlorine was only detected on the MC-treated SAP; however, it was not uniformly dispersed. In contrast to the SAP granules, SEM/EDS showed a uniform distribution of chlorine on the SAFs. The presence of MC was further confirmed by FTIR analysis (see Supporting Information).[16] To ensure that the coating procedure did not negatively impact the SAP granules, the absorptive capacity of the MC-treated SAP granules was compared with the control SAP granules. One milligram of SAP and MC-treated SAP absorbed ∼155 and 148 mg of water, respectively. No significant difference was found after treating SAP granules with MC (Figure 2).

The MC-modified granules were tested for their ability to oxidize 3M3MB, a thiol surrogate for sulfur-containing compounds commonly associated with odor in absorbent personal care devices, in both aqueous and organic solutions.[17,18] After 5 min of incubation in aqueous solutions, MC-treated SAP reduced the amount of this thiol by 51.3% (2.2 μmol). At this same time point, MC-treated zeolite and bentonite reduced the amount of detectable 3M3MB by 41.6% (1.3 μmol) and 33.1% (2.0 μmol), respectively (Figure 3B). After 30 min, both MC-treated zeolite and bentonite further reduced the amount of detectable thiol to 64% (2.3 μmol) and 58% (2.5 μmol), respectively. In contrast, MC-treated SAP showed no further modification of 3M3MB after 30 min. MC-

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ACS Omega 2024, 9, 10201−10206

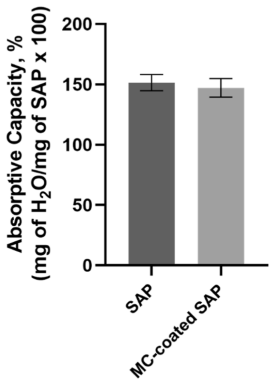

Figure 2. Absorptive capacity of SAP and MC-treated SAP granules. The mass of water absorbed by the granules was determined and Welch's *t*-test applied ($p > 0.05$).

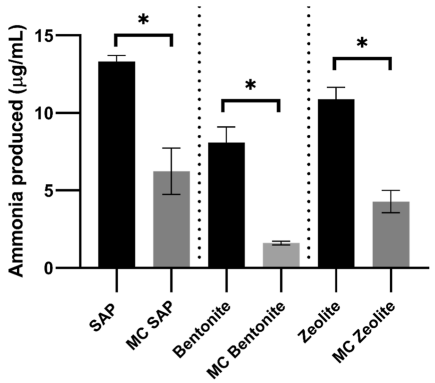

Figure 4. Inhibitory effects of MC-treated granules on urease activity. The amount of ammonia produced by urease was determined by UV−Vis spectrophotometric analysis at 340 nm. The amounts of ammonia produced by the treated samples were significantly different ($p < 0.05$) than the control samples using Welch's *t* tests. Significant differences are indicated by *.

treated SAP produced similar results in ethanol, where the reduction of 3M3MB was ∼2.3 μmol (Figure 3C).

In tests of exoenzyme inactivation, urease was incubated with MC-treated granules for 30 min. MC-treated bentonite was most effective at urease inhibition, reducing the activity by ∼80%. MC-treated SAP granules and MC-treated zeolite decreased the enzyme activity by ∼54 and ∼40%, respectively (Figure 4).

In tests of the antibacterial effectiveness of MC-treated SAP granules against two species biofilms, both *S. aureus* and *P. aeruginosa* exhibited significant declines in CFUs, corresponding to 4.2 and 3.7 log reduction value (LRV), respectively, after 24 h of exposure (Figure 5). SAF nos. 2351 and 2344 were tested in this model and showed 2.8 and 3.7 LRV for *P. aeruginosa*, respectively; values were 3.1 and 3.9 LRV for *S. aureus* (Figure 4).

DISCUSSION

Odor control can be conferred on absorptive media by the introduction of antimicrobial properties in the form of silver nanoparticles and quaternary ammonium salts, and more recently, disinfectants such as sodium dichloroisocyanurate have been mixed with SAP granules.[19−21] However, current

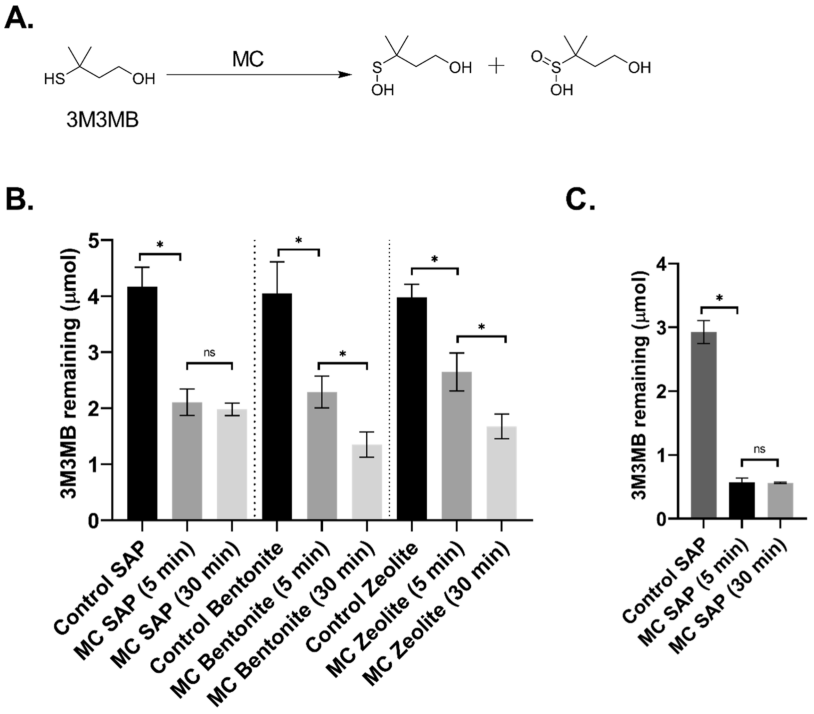

Figure 3. Modification of 3M3MB by MC-treated granules. (A) Structure of 3M3MB and major products identified by LCMS. (B) 3M3MB modification by MC-treated SAP, bentonite, and zeolite in 50 mM MES buffer pH 6.8. (C) 3M3MB modification by MC-treated SAP in ethanol. Welch's *t*-test was applied, and the values for modification of 3M3MB in all treated samples were significantly different ($p < 0.05$) than the modification of 3M3MB in the control samples. The amount of 3M3MB modification in both MC-treated bentonite and zeolite samples incubated for 30 min was significantly different than after 5 min samples ($p < 0.05$). There was no significant difference (ns) in the amount of 3M3MB modification between the MC-treated SAP samples at these same time points ($p > 0.05$). Significant differences are indicated by *.

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ACS Omega 2024, 9, 10201−10206

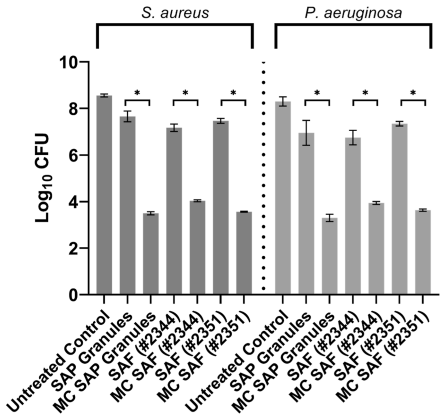

Figure 5. Log_{10} CFU reductions of MC-treated SAP granules and SAFs after 24 h topical application to a two-species biofilm in a chronic wound-like biofilm model. Welch's *t*-test was applied, and the reduction values from all treated samples were significantly different ($p < 0.05$) than the controls. Significant differences are indicated by *.

methods have yet to meet the dual needs of retaining absorptive capacity and scalability for commercial purposes. Independent of the plate-like or spherical granule shape, MC-treated SAP granules conveyed chlorine and retained their full absorptive capacity, requiring no alterations in the amounts necessary for current performance standards. This process is easily scalable, requiring only a solvent evaporation step after treatment.

As antimicrobial resistance continues to increase, strategies that avoid targeting specific cellular mechanisms are increasingly favored to promote longevity of the treatment. For example, silver, which is used in many wound dressings, fosters development and prevalence of silver resistance, leading to diminished antimicrobial efficacy for the cost involved.[22,23] Compounds that confer antimicrobial activity through oxidative chorine (Cl^+) impact multiple microbial targets, making it less likely for antimicrobial resistance to emerge. The direct, widespread effects of Cl^+ on structural and metabolic processes, exoenzymes, and chemical reactivity with microbially produced volatile compounds serve both to reduce microbial load and corresponding malodor.[13] These practical advantages can be expected from industrial deployment of the process used in this study.

MC-treated SAP led to changes in granule performance in tests of malodorant degradation and enzyme inactivation. They also showed antimicrobial efficacy in a mixed-species, chronic wound-like biofilm model. Urease activity was quickly reduced in a time frame that is consistent with the typical use patterns of incontinence garments, which generally involve several hours of contact. The surrogate compound 3M3MB, representative of sulfur-containing malodorants that accumulate in hygiene devices such as methanethiol and dimethyldisulfide, was rapidly modified, and it is likely that this pathway contributes to malodor control.[7,8,18] Optimization of Cl^+ loading will ensure compatibility with the demands of routine use for the range of hygiene devices that rely on SAP granules.

High-level antibacterial efficacy of modified SAFs used in wound dressings would provide a particularly important advantage for the control of both acute and chronic wound infection. Adsorption of MC onto conventional wound dressing fibers has been proposed for this purpose; however, the possibility of combining antimicrobial activity with high absorption of wound fluid offers an attractive means to control two different and difficult-to-manage aspects of chronic wounds.[16] MC-treated SAP granules effectively reduced bacteria in a mixed-species chronic wound biofilm, which comprised the two most commonly coisolated wound pathogens. Mixed-species biofilms are regularly seen in chronic wounds, where they are allied with higher antimicrobial tolerance and increased virulence.[14] Evidence for the control of wound biofilm populations by the incorporation of MC into SAFs suggests that a consequent effectiveness for malodor control could be expected from their use. This would be of crucial benefit to chronic wound and incontinence management in healthcare where odors are known to have a significant and negative impact on the quality of life.[8,9,24−27]

CONCLUSIONS

MC-treated SAP granules show effective antimicrobial activity and malodor control in laboratory test systems. The oxidative chlorine of MC, unlike more common antimicrobial agents, works both as an inactivator of odor-generating microbes and as an effector in other pathways of malodor control. The MC-treated SAP granules provide a novel tool for improving personal hygiene and quality of life. Outside of personal hygiene and clinical use, the use of modified SAP granules in nonwoven food packaging pads and for accidental spill remediation in healthcare settings could also be of value in infection and odor control.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07164.

LCMS data for 3M3MB reactions with MC, IR data for the detection of MC, and SEM/EDS data (PDF)

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https://doi.org/10.1021/acsomega.3c07164
ACS Omega 2024, 9, 10201−10206

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Notes

The authors declare the following competing financial interest(s): JFW serves as technical advisor to MedeSol LLC.

■ ACKNOWLEDGMENTS

L.I.R. received gift funding at the University of Washington Bothell from MedeSol LLC.

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