Economic Assessment and Pathogenic Bacteria Inhibition of Bovine Hide Presoaking Solutions Formulated with Enzymes that can Remove Adobe-type Manure

by

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Abstract

Bovine hide presoaking solutions formulated with crude glycerol and only a quarter of the amount of biocide (such as Proxel-GXL) and surfactant (such as Boron-TS or Busan1009) that the industry is commonly using, have recently been developed and are effective in removing adobe type manure attached to the cattle hide. The goal of this research project was to investigate potential effects of incorporating enzymes that can attack the adobe type manure and could break down adhesion to hide and enhance its removal. If an optimal amount of cellulase or xylanase used individually or in a combination of both was included, lowering the concentration of crude glycerol from 10% to 5% is feasible. From conclusive results, the combination of cellulase and xylanase worked synergistically because a lower concentration of each than when used individually also has demonstrated improvement in manure softening efficiency. The texture analysis of soaked hardened manure showed that the enzymes were quite promising in softening which can be translated to loosening and eventually the enhancement of hardened manure removal. Chlorine dioxide also was incorporated in the formulation and was associated with a reduction in manure odor. The inclusion of sodium hydroxide in the formulation had also enhanced the microbial growth inhibition of pathogenic bacteria that were tested. The cost of implementing the new formulations is similar to those traditionally used by the industry. In addition, the new soaking solutions have a more favorable impact on the environment.

Introduction

The removal of damaging hardened manure prior to curing is desirable for a more effective preservation and for longer storage during shipment of about 33 million bovine hides from USA to countries where it will be processed to leather. The standard presoaking solution has been shown to be effective in inhibiting most of the microbial growth, but not effective in removing adobe type manure.[1, 2] And also, due to the high concentration of biocide and surfactant, it is not considered environmentally friendly. The inclusion of crude glycerol and sodium carbonate proved to be beneficial in enhancing the softening of the hardened manure thus improving the efficiency of its removal.[1, 2] Glycerol is an amphiphile that can insert into surfactant micelles and modify the interfacial tension of water that helps in dissolving the hydrophobic and hydrophilic substances in hardened manure. Crude glycerol and the other components present in it also could have enhanced manure removal efficiency because of its better efficiency compared to pure glycerol.[1, 2] Some inherent microbial growth was found in crude glycerol[1] that is why the inclusion of a relatively environmentally friendly biocide is necessary. Surfactants also are added. Because of the presence of both hydrophobic and hydrophilic regions in these compounds, making it possible for lysis of lipid membranes, solubilization of antigens and washing of various contaminants or complexes.[3-5] Another clear candidate is chlorine dioxide (ClO_2) which is used in sanitizing and cleaning at various food processing stages.[6-8] It is a broad spectrum biocide with 2.6

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times the oxidizing capacity of chlorine across a broad pH range and not as harmful to the operator.[7] ClO_2 has a unique biocidal properties and has been used as an effective disinfectant and algaecide. It has already been approved and used by EPA's Office of Drinking Water (0.8mg/L.)[8] In the rendering, chlorine dioxide controls odors by destroying hydrogen sulfide through chemical oxidation. The use of chlorine dioxide increases sanitation and disinfection and, in the case of food and produce, offers the added benefit of not leaving a residue.[6-8]

Enzymes from many sources and with many different activities and targets have helped the food and feed industries extend shelf life, optimize production, add texture, ensure quality consistency and reduce costs – gains that enabled the customers to reduce their water and energy consumption and waste.[12, 13] The main goal of the research project was to develop an environmentally friendly presoaking formulation (SF) by incorporating enzymes that can act on adobe type manure components and can effectively loosen and eventually remove the manure from its very tight adherence to the hair on bovine hides.

Previous efforts were made to investigate the effects of individual enzymes and enzyme mixtures on manure removal and it was found that mixtures of cellulase, xylanase and especially with laccase (ligninase) were more effective than individual enzyme treatments.[13-15] However, this approach has not been implemented by the hide industry because it was neither practical nor cost effective. The inclusion of ligninase called for a rather complicated and labor intensive enzyme production and preparation. It took about 10-20 days to harvest the full-grown fungi and few more days for the isolation and preparation of the enzyme.[14, 15] In this paper, the use of enzymes with the incorporation of crude glycerol, sodium carbonate and chlorine dioxide with very low concentration of biocide and surfactant were investigated. The inclusion of the ingredients used in oxidative unhairing such as sodium hydroxide and sodium percarbonate were also tried at lower concentrations. The goal was to enhance the efficiency of the previously promising presoaking formulation (SF) for removal of adobe type manure removal from the hair on bovine hides. The cost assessment was conducted to determine the economical feasibility to implement the more environmentally friendly soaking formulation(s).

EXPERIMENTAL

Materials

All the chemicals used for the conventional processes are of commercial grade. The chemicals used for the preparation of the different soaking formulations are of analytical grade. The Crude Glycerol was obtained from Griffin Industries (Butler, KY) and was used as received. The different components present in the crude glycerol were found to be ~77% glycerol, 0.5% methanol, 0.4% other organic materials including fatty acids and ~22% water. The chlorine dioxide used is commercially sold as AquaDry3000 from Beckart Environmental, Inc (Kenosha, WI.) The industrial enzymes are courtesy of Thomas Shaughnessy and Mark Emalfarb from Dyadic International, Inc. (Jupiter, FL.) The biocide either Proxel-GXL from Lonza (Basel, Switzerland) or Busan 1009 from Buckman, Inc. (Memphis, TN); Boron TS was from Rohm Tech., Inc. (Malden, MA.) Fresh hides with full of adobe type manure were obtained from a local beef cattle processing plant (JBS, Souderton, PA).

Manure Soaking and Softening Experiments

Since the softening of the hardened manure is key to its removal during the demanuring process, the experiments presented in the previous paper [1,2,5] and in the current study, were designed such that the changes in the "hardness" of the manure balls were monitored. The texture changes were tracked over a specified period of time (30 min to 2 h span) in triplicates for the Texture Profile Analysis.[1, 5] The manure samples were selected according to similar physical characteristics such as size, weight, and hardness and taken from the same animal. The sample was analyzed for texture at an initial reading (To = 0 sec) without soaking formulation added. Each manure sample was immersed in the designated formulation placed and sealed in a 4 x 10.2 cm Ziploc polyethylene (PE) bag. On average, about 200% (v/w) SF was needed to completely immerse the hardened manure sample. Then the sample in solution was agitated on a Gyrotatory ® Shaker - Model G2 at approximately 120 RPM. Texture analysis reading was performed using the CT3-texture analyzer at 30 min and 60 min after soaking in the respective solution to determine the amount of work needed to attain the same deformation at peak load, equivalent to the percentage of softening of the hardened manure as previously reported.[1, 5]

Antimicrobial Activity Against Pathogenic Bacteria

The preliminary antimicrobial activity screening of the hide presoaking formulations against pathogenic bacteria were performed using four (4) pathogenic bacteria. Four pathogenic bacteria, three Gram-negative and one Gram-positive, *Escherichia. coli* O157:H7 ATCC 43895, *E. coli* non-O157 STEC serotype O26:H11, MDR *Salmonella* Typhimurium DT-104, and *Listeria monocytogenes* 1/2b were tested, respectively. Each strain was grown statically in nutrient broth (Becton Dickinson, Sparks, MD) for 16-18 h at 37°C. Cell concentrations of each strain were adjusted using a spectrophotometer (Thermo Electron Corp., Madison, WI) at 600 nm to approximately 10^8 CFU/ml. The cell concentrations of each strain were further diluted using maximum recovery diluents (Becton Dickinson) to the final concentration of 10^6 CFU/ml. Each strain was streaked on Mueller-Hinton agar (Becton Dickinson) using a sterile cotton swab to make a lawn of each pathogen. Four sterile blank discs, (6 mm; Becton Dickinson) which three discs for each soaking solution and one disc for control negative) were aseptically placed on the agar plate containing each pathogenic bacterium. An aliquot of 25-μl of each presoaking solution was placed on the discs (control negative using sterile water) and let the plate stood at room temperature until all liquid was absorbed. Plates were incubated at 37°C for 16 h and the zones of inhibition around the discs were observed and measured for diameter (mm.) The average of zone of inhibition from three discs for each pathogen and each soaking formula was reported.

Tannery Scale Application Protocol for Manure Removal
Fresh hides saturated with adobe manure from a local meat packing plant in Souderton, PA were collected and split down the back into left and right segments. Segmented hide samples were soaked in drums for 3 hours calculated at 150% float of presoaking formulation. The determinations were only qualitative because the relative amount of removed manure was compared visually to the efficiency of each other and no statistical analysis was done. Samples were removed from the drums for observation and tracking of manure removal. Samples were then washed, tanned and processed into leather for mechanical property testing.

RESULTS AND DISCUSSION

The standard soaking formulation with high concentrations of biocide and surfactant completely hindered general microbial growth of the manure washings on LB agar plates.[1] Whereas, the soaking formulations with only a fraction of the standard solution but supplemented with crude glycerol and sodium carbonate prevented about 70% of the microbial growth.[1] When chlorine dioxide of about 200ppm was previously added to the formulation, almost all microbial growth were eliminated.[1]

Manure Soaking and Softening Experiments
Texture analysis was performed using the CT3-texture analyzer (at 0 min) before soaking and at 30 min and 60 min after soaking in the respective solution to determine the amount of work needed to attain the same deformation at peak load which is equivalent to the percentage of softening of the hardened manure. 2, 5 In the literature, the optimal pH of the enzymes, cellulase and xylanase are in the range of weakly acidic at 5 to neutral pH of 7. 12, 13 However, when the formulations were used with unadjusted pH of about 10.5 to 11, the softening was more efficient than when the pH was adjusted to around pH 5 which was supposedly the optimal pH of the enzymes. As a concrete example, it was observed that the formulation with cellulase at pH of about 10.6, the softening after 30 min soaking is about 87%. Whereas after adjusting the pH to around 5, a significant reduction in softening efficiency to only about 12%. Similar results were observed for the formulation with xylanase at pH ~10 that showed 83% softening compared to only ~42% after adjusting the pH to ~5. The same trend was observed for the 60 min softening trials. When high pH alone due to the presence of NaOH was verified, the softening effect was not as efficient (60-77%) as when other ingredients and enzymes were also present (80-95%). Whereas with water alone, it has shown only about 33-38% softening. Additional softening trials were performed using a new set of formulations with the unadjusted pHs (in most cases pH range of 10-11) as shown in Table I.

Upon addition of 10% crude glycerol and 1% sodium carbonate to just 25% standard presoaking solution, the efficiency in softening of the hardened or adobe type manure after 30 to 60 min was improved significantly to about 80-90% in SFz-2 compared to 74-81% with 100% standard SF in SFz-1 (Table 1). The addition of about 0.02% (200ppm) cellulase enhanced the softening to about 86% after presoaking for 30 min compared to 80% in SFz-2 without cellulase. The softening was efficient at ~84% when xylanase was used at 500 ppm even with only 5% crude glycerol for SFz-7 compared to 50% efficiency if 200ppm xylanase was used with 10% crude glycerol in SFz-6. With the inclusion of about 20 to 200 ppm chlorine dioxide, the softening efficiency of 0.02% (200 ppm) xylanase was improved from 50% to ~77% as seen in SFz-9 compared to SFz-6 (Table I). In SFz-10, a synergistic effect of combining lower concentrations of the two enzymes (200ppm of cellulase and 200 ppm of xylanase) was observed even with lower concentration of crude glycerol at 5%, such that the softening improved significantly over that of SFz-5 (75 to 87%). The higher concentration of chlorine dioxide at 200 ppm in formulations prepared for Table I appeared to have inhibitory effect on the enzyme activity compared to the lower concentration of 20 ppm used in previous formulations (unreported data).

The t-test values between the 30 and 60 min soaking were calculated using the equation illustrated in the previous manuscript.2 The bold t-test values signified that there was significant further softening taking place when the manure sample was allowed to soak for additional 30 min. For the plain font t-test values, the softening was rather fast. The additional softening after 60 min of soaking was not significant because the manure was already softened enough after just 30 min. These fast acting formulations, SFz-3, SFz-7 and SFz-10 are among the desired ones because shorter time is required to effectively soften and eventually remove the tightly bound hardened manure from the bovine hides.

To find out if the newly developed presoaking formulations have any adverse effects on the quality of leather, the presoaked hides were processed to leather in the same way as the traditional presoaked hide. The different promising formulations were prepared and the corresponding ingredients are illustrated in Table II. The mechanical properties were then determined using the ASTM D2813 method, a Standard practice for sampling leather for physical and chemical tests.

TABLE I
Measurement of softening efficiency of manure samples.

SF code	Ingredients of different presoaking formulations	30min: % softening	60 min: % softening	t-test values 30 - 60 min
SFz-1	100% std SF (0.1%Proxel+0.15%Boron-TS)	74.0 ± 3.3	81.0 ± 2.4	**3.8**
SFz-2	25% std SF(0.025%P+0.0375%B-Ts) + 10% CG + 1% SC	80.0 ± 2.9	90.0 ± 3	**5.4**
SFz-3	**25% std SF + 1% SC+ 10% CG+ 200 ppm cellse**	**86.5 ± 3.2**	**89.0 ± 3.8**	**1.3**
SFz-4	25% std SF + 1% SC + 5% CG + 200 ppm cellse	49.1 ± 3.7	80.4 ± 4	**12.8**
SFz-5	25% std SF + 1% SC + 5% CG + 200 ppm cellse + 200 ppm xylns	68.5 ± 4	75.0 ± 3.2	2.8
SFz-6	25% std SF + 1% SC + 10% CG + 200 ppm xylns	42.2 ± 2.9	50.0 ± 3.6	**3.8**
SFz-7	**25% std SF +1% SC + 5% CG + 500 ppm xylns**	82.6 ± 3.4	84.0 ± 4	0.6
SFz-8	25% std SF + 1% SC + 5%CG + 200 ppm cellse + 200 ppm ClO_2	68.0 ± 2.4	72.5 ± 3.3	2.5
SFz-9	25% std SF + 1% SC + 5% CG + 200 ppm xylns + 200 ppm ClO_2	74.6 ± 3.4	76.7 ± 4	0.9
SFz-10	**25% std SF + 1% SC + 5% CG + 200 ppm xylns + 20 ppm ClO_2 + 200 ppm cellse**	82.0 ± 4	87.0 ± 5	2.8

CG = crude glycerol, std SF = standard soaking formulation (0.1%Proxel+0.15%Boron-TS);
ClO_2= chlorine dioxide; cellse = cellulase; xylns = xylanase; SC = sodium carbonate;

TABLE II
Formulations prepared for large scale and tanning trials at the tannery.

Trial #	Ingredients in the soaking formulations
SF-M#1	25% Std SF + 5% CG+ **2.5% NaOH + 1% SperC + 0.15% Alk protease**
SF-M#2	25% Std SF + 5%CG + 2.5% NaOH + 1% SperC + 0.05% Cellulase
SF-M#3	25% Std SF + 5%CG+1% SC+**1%NaOH + 0.025%Cellulase+ 0.025%Xylanase**
SF-M#4	25% Std SF + 10% CG + 2% SC + 0.25% Alk Protease
SF-M#5	25% Std SF + 10% CG + **2% NaOH + 2% SC + 0.10% Cellulase**
SF-M#6	control, 100% std SF (0.1% Proxel-GXL) + 0.15% Boron-TS)

Std SF = standard soaking formulation; CG = crude glycerol; SperC = sodium percarbonate;
SC = sodium carbonate; Alk = alkaline

The crust leather obtained from differently presoaked hide samples had shown comparable physical and mechanical properties to the traditionally presoaked control hide. SF-M#1, SF-M#3 and SF-M#5 that contained ~2% NaOH, gave relatively more superior quality leather with improved elongation and softness. Whereas the SF-M#4, with higher concentration of alkaline protease (0.25%) compared to 0.15% in SF-M#1 was relatively tougher, had inferior elongation and softness than the control. A smaller amount can go a long way.

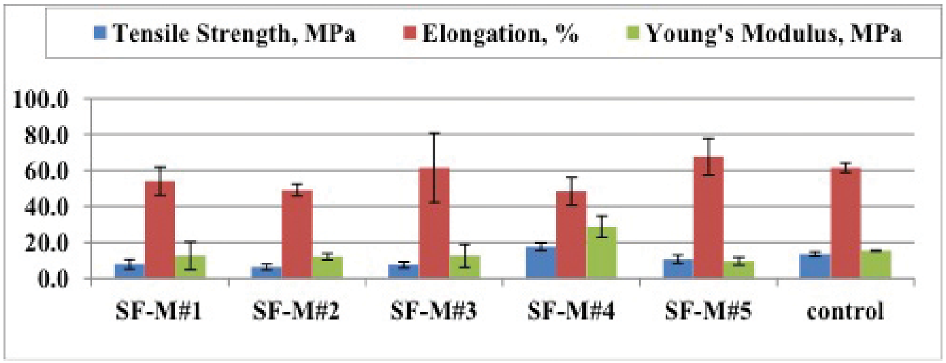

Figure 1. Mechanical Properties of the leather obtained from the 6 choice soaking formulations given in Table II.

Figure 2. Fresh steer hide with adobe type manure samples before soaking.

Figure 3. The left and right regions of the steer hide in figure 2 after soaking and removal of the hardened manure.

Tannery Pilot Plant Scale Protocol for Manure Removal

During the tannery scale application, the fresh steer hide with manure samples as shown in Figure 1 was used. It was observed that the adobe-type manure were ball-like, quite hard, heavy and damaging to the hide grain. The weight alone appeared to be detrimental to the integrity of the hide grain. The manure removal efficiency was determined qualitatively because the relative amounts of manure removed were compared only visually to each other and that is why no statistics could be derived.

The left region was pre-soaked in 100% std soaking formulation (0.15% Boron TS; 0.1% Proxel-GXL.) The right region was pre-soaked in 25% std SF (0.0375% Boron TS; 0.025% Proxel-GXL) + 5.0% C-G + 1.0% Na_2CO_3 + 100 ppm Cellulase. During this pre-soaking procedure both hide samples coiled while in separate drums leaving the manure removal results inconclusive. However, the manure elimination was still effective at ~60% (left region) with the control 100% std SF compared to about 70% (right region) in the newly developed formulation using only a fraction (1/4) of the biocide and surfactant but with crude glycerol and sodium carbonate. In another parallel experiment, about 70% manure removal was observed in the promising soaking formulation composed of 25% standard SF +10% CG + 1% SC and 200 ppm ClO_2. Significant manure smell reduction was observed when chlorine dioxide was added.

When the promising presoaking formulation was incorporated with lower concentration of the ingredients employed in oxidative unhairing technique such as sodium hydroxide and sodium percarbonate, the manure removal was more efficient. The manure that was tightly bound to the hair came along when the long hair was removed from the hide. The short hair and stubbles left became relatively easier and faster to remove during the unhairing step when processing the hides to leather.

Pathogenic Bacterial Growth Inhibition

Due to the high concentration of the biocides used, it was not surprising that the traditional standard formulation was observed to be efficient in inhibiting any growth of the microbes present in the manure washings.[1] The microbial growth inhibition in previous study[1] was performed by inoculating the diluted manure washes on LB agar plates. It showed that by using only 50% concentration of the ingredients, the microbial inhibition efficiency was quite good at ~80%. When only 25% of the standard soaking formulation was used, about 60% inhibition efficiency was still observed.[1] In this paper, the pathogenic bacterial growth inhibition screening was performed to see if the formulations would have some anti-pathogenic bacterial activity. The different formulations relevant to the present study are shown in Table III and the corresponding results are tabulated in Table IV.

Figure 4. Large/pilot tannery scale adobe type manure removal of before and after soaking.

With the addition of ~1% sodium carbonate (Na_2CO_3), the pH of the formulations changed dramatically to ~10.6 from neutral pH. All the formulations were tested for zone of inhibition in its "as is" pH when all the ingredients were mixed. It was found that at higher pH, the zone of inhibition to be more efficient in manure softening and even more enhanced in the presence of the enzymes and NaOH.

The results showed that the formulations SFp-1 and SFp-2, composed of only half (50%) of the concentration of biocide and surfactant than the traditionally used in hide industry, inhibited *E. coli* O157:H7, non-O157 STEC strain O26-H11, *S.* Typhimurium, and *L. monocytogenes strain 1/2b*. The presoaking formulation with only 25% of the std SF still showed considerable inhibitory effect against the pathogens tested as in formulation SFp-3 and even in the presence of crude glycerol and sodium carbonate as in formulation SFp-4. The formulation SFp-8 in Table III (composed of 25% std SF + 5% CG + 1.0% SC + 0.025% Cellulase + 0.025% Xylanase) that gave high% efficiency in softening, did not show zone of inhibition for *E. coli* O157 and O26-H11, but quite potent towards *L. monocytogenes* strain 1/2b.

TABLE III
Different presoaking formulations used for pathogenic bacterial inhibition screening.

PreSoaking Formulation	**Ingredients** (std SF: standard presoaking formulation, CG: crude glycerol, SC: sodium carbonate, SperC: sodium percarbonate)	pH
SFp-1	50% std SF	7.7
SFp-2	50% std SF + 1.0% SC	11.1
SFp-3	25% std SF	7.2
SFp-4	25% std SF + 10% CG + 1% SC	10.9
SFp-5	25% std SF + 5% CG + 2%NaOH + 1%SperC + 0.15% Alkaline Protease	13.4
SFp-6	25% std SF + 5% CG + 2%NaOH + 1%SperC + 0.05% Cellulase	13.4
SFp-7	25% std SF + 5% CG + 2%NaOH + 1%SperC + 0.05% Xylanase	13.4
SFp-8	25% std SF + 5% CG + 1%SC + 0.025% Cellulase + 0.025% Xylanase	10
SFp-9	25% std SF + 5% CG + 2%NaOH + 1%SperC + 0.025% Cellulase + 0.025% Xylanase	13.4

The presoaking formulations with 2% sodium hydroxide and at high pH of ~13.4 as in SFp-5, SFp-6, SFp-7 and SFp-9, have exhibited good pathogenic bacterial growth inhibition across the board with respect to all the strains used in the trials. Formulation SFp-8 is similar to SFp-9 except that SFp-8 does not contain 2% NaOH. SFp-8 does not inhibit E. *coli* O157 and E. *coli* O26-H11 but more potent towards Listeria *LM* 1/2b strain. In another occasion, SFp-8 appeared to be more selective by inhibiting only E.Coli O145:NM among the five E. coli strains tried and also inhibited growth of S. Newport strain (unreported data.) Thus the presence of cellulase and xylanase must have contributed some bacterial growth inhibition in addition to the presence of sodium hydroxide.

Cost Assessment

The amount of ingredients required in preparing the 150% float meant that for every 1000 kg of hides, 1000 kg (or 1000 li final volume) of the presoaking solution was needed as shown in Table V. If one hide weighed about ~50kg, then 1000 kg would be around 20 hides. The price of the chemicals was based on bulk purchases. The surfactant used was BoronTS and were sold for $1.25/ Lb in 450 lbs drum. A 10% discount could be possible if delivered in railroad cars or trucks. The biocide used was Proxel GXL which was sold at about $5.93/lb for 2 - 7 drums batch but the bulk sale price was about $5.93 / lb for 441 lbs/drum. Proxel GXL can be replaced with Busan 1009 sold at $6.34 / pound per in 440 lbs drum. Crude glycerol, was obtained at $0.32/lb for 5 gallon batch from Griffin Industries of KY, but could get it for $0.065/lb when bought in bulk quantity from the biodiesel plant. Sodium carbonate technical grade was selling for $275/ton from FMC Corp. Chlorine Dioxide Aquapulse Sytems sell an onsite ClO_2 generator system and their estimate (w/ adjustments) had an approximate price of $18.35 / lb of ClO_2. The price for one kit yielding 0.25 lb of ClO_2 was obtained from Beckhart Environmental at about $35.00. Dupont price for truckload quantity of sodium chlorite would be about $4.00/lb of ClO_2 produced. For Cellulase and Xylanase, the price of ~$10.00/kg or $4.52/ lb was used. Sodium Hydroxide was approximated at $0.50/kg based on $500 per metric ton or 2200lbs in bulk from Alibaba.com, a Global Trader company. When NaOH is used at 2% in 150% float, the amount in 1000kg hides will be $22. Water was priced at approximately $20.00 / 1,000 cubic feet or per 62,400 lbs thus the price of water per hide is almost negligible and uniform in all formulations.

Based on the cost per one hide from Table IV, the newly developed formulations without inclusion of NaOH are relatively cheaper compared to the standard presoaking formulation. There are numerous valuable advantages to implementing the newly developed formulations. The overall chemical costs are reduced, sewage disposal costs could be lowered or even eliminated, and system deterioration would be diminished. With NaOH, the price is almost doubled as in PSF-Z6 to Z8. But considering the benefits of significant efficiency in manure removal and pathogenic bacterial growth inhibition, the increase in price is still reasonable. Likewise, when 1% sodium carbonate is replaced by 1% sodium percarbonate in the formulation, the price contribution would be approximately the same.

TABLE IV
Pathogenic growth inhibition of formulations illustrated in Table III.

PreSoaking Formulation	Escherichia Coli		Salmonella	Listeria
	0157	*026*	*Typhimurium*	*LM 1/2b*
SFp-1	12	18	12	17.5
SFp-2	10	15	11	15.5
SFp-3	8.5	8.5	6.5	9.5
SFp-4	8	12	6	10
SFp-5	10.5	9.5	11	10
SFp-6	10.5	9.5	11.2	11
SFp-7	10.2	10	10.8	10
SFp-8	0	0	0	15
SFp-9	10.5	10	10.5	11

TABLE V
Cost assessment of newly developed presoaking formulations.

Ingredient	Boron TS	Busan 1009	Crude Glycerol	Sodium Carbonate	Chlorine Dioxide	Xylanase	Cellulase	Sodium Hydroxide	1000 kg of hides	50 kg/hide
$ price/kg	$2.67	$13.97	$0.07	$0.30	$18.40	$10.00	$10.00	$0.50	~20 hides	per hide
PRE-SOAKING FORMULATION COST per 1000 kg hide with 150% float										
Std PSF	$4.13	$13.97	***	***	***	***	***	***	**$18.81**	**$0.94**
PSF-Z1	$1.03	$3.49	***	***	***	***	***	***	**$5.23**	**$0.26**
PSF-Z2	$1.03	$3.49	$14.33	$3.03	$8.09	***	$2.00	***	**$32.60**	**$1.63**
PSF-Z3	$1.03	$3.49	$14.33	$3.03	***	$1.00	$2.00	***	**$25.51**	**$1.28**
PSF-Z4	$1.03	$3.49	$7.16	$3.03	***	$2.00	$2.00	***	**$19.38**	**$0.97**
PSF-Z5	$1.03	$3.49	$7.16	$3.03	$8.09	***	$2.00	***	**$25.47**	**$1.27**
PSF-Z6	$1.03	$3.49	$7.16	$3.03	***	***	***	$22.00	**$36.71**	**$1.84**
PSF-Z7	$1.03	$3.49	$7.16	$3.03	***	$1.00	***	$22.00	**$37.71**	**$1.89**
PSF-Z8	$1.03	$3.49	$7.16	$3.03	***	$2.00	$2.00	$22.00	**$40.71**	**$2.04**

CONCLUSIONS

Incorporation of the enzymes such as cellulase and xylanase and the lower concentration of crude glycerol have proven to give a more economical and eco-friendly presoaking formulations. It is possible to lower the concentration of crude glycerol (from 10% to 5%) and still exhibit efficient hardened manure removal if enzymes and chlorine dioxide are incorporated in the formulations. The synergistic effect of the combination of cellulase and xylanase proved to be more efficient in softening the hardened manure at lower concentrations. Indeed, the enzymes are quite promising in softening which can be translated to loosening and removal of the damaging adobe type manure from bovine hides. When the promising presoaking formulation was incorporated with lower concentration of the ingredients employed in oxidative unhairing technique such as sodium hydroxide and sodium percarbonate, the manure removal became more efficient. The added benefit that NaOH contributed is the enhancement in pathogenic bacterial growth inhibition efficiency of almost all the strains that commonly cause serious human diseases that can even lead to death. The presence of cellulase and xylanase have also contributed to some bacterial growth inhibition particularly towards Listeria *LM* 1/2b. Considering the low cost in the procurement of crude glycerol, it is quite desirable as an ingredient in the new presoaking formulation. Significant manure smell reduction and additional microbial inhibition are observed when a user friendly chlorine dioxide also was added. The presence of even just 25% of the concentration of biocide and surfactant normally used by the hide industry, had shown microbial growth inhibition. The outcome of this research is valuable to the hides and leather industry to improve the safety of the handlers in the processing of hides and the quality of the leather derived from it. It also has potential for food safety applications when the new formulations would be applied to live or newly stunned cattle during harvest. It would also be interesting to pursue the food safety application on produce by verifying if the manure collected after presoaking in the new SFs will not be detrimental if used as organic fertilizer.

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