

SOAKING FORMULATIONS THAT CAN SOFTEN HARDENED BOVINE MANURE

by

MILA ALDEMA RAMOS*, ZERLINA E. MUIR, RICHARD D. ASHBY AND CHENG-KUNG LIU
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center
600 E. MERMAID LANE, WYNDMOOR, PA 19038, USA

ABSTRACT

Many of the damaging problems in hides and leather industry, such as grain damage and weakening of the leather product, can originate from putrefaction due to microbial contamination and mechanical stress due to the heaviness and pressure of hard to remove adobe type manure on bovine hides. New washing methods are urgently needed to effectively clean raw hides for their storage and shipments. The amphiphilic property of detergents and the capability of glycerol to insert into the detergent micelles were investigated in the formulation of an ideal washing solution. Since the softening of the hardened manure is the key to its removal during the demanuring process, the initial experiments presented in this report were designed such that the changes in hardness of the manure balls were monitored using a texture analyzer. The work needed to cause the same amount of deformation on the manure sample was measured and compared before and after soaking in the respective formulations. Various detergent types such as anionic (SDS), nonionic (Tween 20 and TritonX-114) and zwitterionic (LDAO) are evaluated and compared to the biodegradable surfactant, sophorolipid (SL). The sophorolipid with the addition of recycled crude glycerol was observed to be more effective in softening the hardened manure samples and will be further studied for "adobe" type manure removal.

RESUMEN

Muchos de los problemas en daños en las pieles y consecuentemente en la industria del cuero, tales como daños a la flor y su debilitamiento en el cuero producido, pueden provenir por la putrefacción debida a contaminación bacteriana y distorsiones mecánicas debidas al peso y presión causada por el adobamiento del estiércol adherido a las pieles bovinas. Nuevos métodos de lavado son urgentemente requeridos para efectivamente limpiar las pieles crudas para su almacenamiento y envío. La característica anfifílica de los detergentes y la capacidad de la glicerina en insertarse dentro de las micelas formadas por los detergentes fueron investigadas en la formulación de una solución ideal para el lavado. Ya que el ablandamiento del estiércol endurecido es clave en su mismo proceso de remoción, los experimentos iniciales en este informe fueron diseñados tales que los cambios en la dureza en las bolas de estiércol fueron monitoreados por medio de un analizador de textura. El trabajo requerido para causar la misma cantidad de deformación en la muestra de estiércol se determinó y se comparó antes y después de remojar en las respectivas formulaciones. Varios tipos de detergentes tales como aniónicos (SDS), no-iónicos (Tween 20 y Triton X-114) así como zwitteriónicos (LDAO) fueron evaluados y comparados con un detergente biodegradable, Sophorolipid (SL). El Sophorolipid con la adición de glicerina recuperada, se observó ser el más efectivo en el ablandamiento de las muestras y será investigado en un futuro para usarse en la remoción de estiércol adobado.

*Corresponding author e-mail address: mila.ramos@ars.usda.gov.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
Manuscript received September 30, 2010, accepted for publication March 1, 2011

INTRODUCTION

Animal hides are one of the largest agricultural export commodities of the US and are valued at over \$2.2 billion annually.¹ However, the poor quality of hides delivered to the tanner is a perennial problem, especially when the value of hides is dramatically reduced because of putrefaction.² These damaging effects are usually due to ineffective hide preservation, microbial contamination and the mechanical stress due to the heavy and hard to remove adobe type manure adhering to bovine hides. Manure in hides has been the source of leather quality deterioration and in the lowering of the grades and values of raw hides. The phenomenon of hardened or “adobe” type manure starts in January and lasts into May when the animals are overcrowded in the feedlots. Removal of these manure balls as early as possible in the processing of the hide is desirable for several reasons.³ To avoid contamination of meat, they should be removed prior to removing the hide from the carcass. To assure the best return to the hide dealer, they should be removed prior to curing. To avoid hide damage in the form of large holes, they must be removed prior to fleshing.

For many years, various approaches were attempted including mechanical or enzymatic treatments, but currently there is no truly effective method adopted by the industry to deal with this problem. 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 laccase (ligninase) were more effective than individual enzyme treatments.⁴ However, the production of this enzyme mixture, particularly the inclusion of ligninase, was rather complicated and lengthy, taking about 10-20 days prior to harvesting the full grown fungi and few more days for the enzyme isolation and preparation.⁵ Various unpublished techniques³ were experimented by the industry, such as using fresh water raceways where one might expect that the mechanical action of water with a minimum contact time (~1 h) could soften the manure to the point of removal without tearing the grain. Results were not satisfactory and the process was very labor intensive and created an effluent nightmare. Slaughtering with hot water hair removal, much like the scalding of porcine animals, was also investigated but the temperature required could not guarantee the grain or fibrous integrity of the hide would not be damaged. Industry also experimented with mechanically “crushing” the manure prior to defleshing or demanuring. Some attempts were to add chemicals to this process; however, the efficiency of manure removal was poor. Further more, when dealing with fresh hides, one has to be aware of the consequences that these products may have in the rendering and wastewater stream. Additionally, the costs tend to be worse than the gain.³

The polarity of the solvent is a crucial factor in the dissolution. Therefore, one may expect that glycerol and biosurfactants will have potential applications in eco-friendly manure cleansing formulations. Glycerol is commonly found in various cleaning formulations, such as shampoos, toothpastes, lotions, body washes and hand soaps. Due to its amphiphilic ability of inserting into the surfactant micelles; glycerol has been used successfully in preservation and crystallization of water insoluble membrane proteins in their soluble and native state by lowering the activity of the water in the sample.⁷ In our current research project, glycerol will be incorporated in cleaning and hide preservation formulations and may provide a desirable new outlet for the large amounts of glycerol produced as a byproduct by the growing biodiesel industry.⁸ The biodegradable surfactant, a sophorolipid,⁹ is evaluated with and without glycerol for manure removal and compared to results obtained from commercial detergents for comparable potential manure removal formulation. Surfactants are amphiphilic agents that modify the interfacial tension of water.¹⁰⁻¹² They include soaps, detergents, emulsifiers, dispersing and wetting agents, and several groups of antiseptics. The presence of both hydrophobic and hydrophilic regions make these compounds useful for lysis of lipid membranes, solubilization of antigens and washing of various contaminants or complexes. The critical micelle concentration (CMC) values are a guide to detergent hydrophobic binding strengths. The higher the CMC, the weaker the binding and the easier the removal of the detergent, adding salt or electrolytes will lower the CMC and raise the micelle size.¹⁰⁻¹²

The amphiphilic property of detergents and the capability of glycerol to insert into the detergent micelles have been exploited in the formulation of an ideal washing solution. Since the softening of the hardened manure is the key to its removal during the demanuring process, the initial experiments presented in this paper were designed such that the changes in the hardness of manure balls were monitored using a texture analyzer. The work needed to cause the same amount of deformation on the manure sample was measured and compared before and after soaking in the respective formulations.

The other surfactants we used in this study were representative of the different types.

Lauryldimethylamine-N-oxide (LDAO) is a Zwitterionic detergent,¹³ thus it can exist as a nonionic or cationic (protonated) species depending on the pH of the aqueous solution. It has a molecular weight of 229.4 g/mole and a critical micelle concentration (CMC) of 0.023% (w/v). In its nonionic state, the CMC is 1.6 mM, as a mixed micelle it is ~1mM and in its cationic state it is ~2 mM. At higher salt concentrations, the CMC of the three different forms is reduced by about 65-75%. LDAO has been used as a detergent,

emulsifier, wetting agent, foaming agent, softener, milling agent and dyeing auxiliary.¹³

The nonionic detergents Tween 20 and TritonX-114 were also evaluated and discussed in this report. TritonX-114 has a molecular weight of 537 g/mole as a monomer and ~92,000 g/mole as a micelle. Its CMC is ~0.033% (w/v) or 0.2-0.9mM. Whereas, Tween 20 has a molecular weight of 1128 g/mole as a monomer and ~62,000 g/mole as a micelle. Its CMC is 0.06-0.07% in water at room temperature. Tween 20 is a registered trademark and is synonymous to Polyethylene glycol sorbitan monolaurate solution.¹²

Sodium dodecyl sulfate (SDS), also commercialized as sodium lauryl sulfate (SLS), is a strong anionic detergent used in many cleaning and hygiene products. It has a molecular weight of 289 g/mole as a monomer and ~18,000 g/mole as a micelle. Its CMC is 7-10mM or 0.23% (w/v). The SDS molecule has a tail of 12 carbon atoms, attached to a sulfate group, giving the molecule the amphiphilic properties required of a detergent.¹¹

The biodegradable surfactant we used is a sophorolipid (SL) that was synthesized by ARS-ERRC scientists. SLs are extracellular glycolipids that are produced primarily by yeasts of the genus *Candida* (primarily *Candida bombicola*).^{8,9} They are generally composed of a disaccharide (sophorose; 2-*O*- α -D-glucopyranosyl- β -D-glucopyranose) and a hydroxy fatty acid tail that is linked through a hydroxyl group. The fatty acids are generally 16 to 18 carbons in length and may be saturated or unsaturated (for chemical structure of oleic acid-based sophorolipids see Ref 9). Because of their amphiphilic qualities, SLs have reportedly been used as surfactant additives in shampoos and body washes.¹⁴ Because of their large production capacity (reportedly up to 400 g sophorolipid per L of culture batch)¹⁵⁻¹⁶ and the ability to synthesize these materials from cheap feedstocks, SLs have gained interest as potential substitutes for synthetic surfactants in additional applications.

EXPERIMENTAL

Materials and Methods

The fresh dirty hide samples were collected from the local meat packing company, courtesy of JBS of Souderton, PA. The hide areas that did not have visible manure were cut and discarded. Loosened pieces of hardened manure of uniform sizes, similar visible physical characteristics or texture and feel were collected from the dirty hide. The dimensions of each manure sample, such as thickness or width and the length, were taken by using a PRO-MAX Fowler utility digital caliper (Fow74-200-777). For added dryness and to mimic the hardened manure qualities, the manure samples were vacuum dried overnight (~20 hours). The rest of the dried manure samples were covered tightly and kept in a freezer for future

experiments.

The different washing formulations were prepared as follows: The concentration of each detergent is based on its critical micelle concentration (CMC) value. The efficiency of the detergent is improved when all the monomeric molecules are in a micelle form. Thus concentration above the CMC (~ 2 times CMC) were calculated and used.

The following were the 9 different washing formulations tried:

1. 10% pure glycerol (from Sigma), pG
2. 10% crude glycerol (from United Biodiesel Company), cG
3. 1% sodium carbonate from Sigma-Aldrich, SC
4. 1% sodium carbonate (SC) + 10% crude glycerol (cG)
5. 0.1% sophorolipid (SL, ERRC labs) + 1% SC + 10% cG
6. 0.06% Triton X-114 + 1% SC + 10% cG
7. 0.21% Tween20 + 1% SC + 10% (cG)
8. 0.04% LDAO + 1% SC + 10% cG
9. 0.35% SDS + 1% SC + 10% cG

Protocol developed for washing with the pre-prepared surfactant formulations.

1. Weigh the hardened manure pieces individually.
2. Add 200% (v/w) float using the prepared formulation. (For 5g manure, add 10 ml of the desired soaking formulation).
3. Using the CT3 texture Analyzer (Brookfield, King of Prussia, PA), with TA40 stainless cylindrical probe (4.5 mm diameter and 20.5 mm length), measure the corresponding work needed and final load in grams to cause the same deformation at peak of 5 mm and a uniform trigger amount of 10g on each presoaked manure sample.¹⁷
4. Replace the manure into each formulation and agitate or shake gently at room temperature for 2 h.
5. At the end of 2 h, carefully fish out the manure sample from each washing container.
6. Measure the corresponding work (in mJ) and final load (in g) needed to cause the same deformation at peak of 5 mm on each soaked manure sample, at t = 2h.
7. Continue the soaking and gentle shaking at room temperature for another 2 h.
8. At the end of 4 h soaking, carefully fish out the manure sample from each washing container.
9. Measure the corresponding work needed and final load in grams to cause the same deformation at peak of 5 mm on each soaked manure sample, at t = 4h. If the manure has disintegrated during soaking, a quick spin of ~ 4,000 RPM at room temperature for 5-10 min in a centrifuge is required. All the samples should be subjected to the same amount of spin before the measurements in CT3-texture analyzer is done.
10. Tabulate all results and compare the data by plotting the bar graphs.
11. Calculate the ratio and percentage of work needed to cause the uniform deformation at peak of 5mm into the manure sample after soaking compared to the initial work needed in each respective formulation.

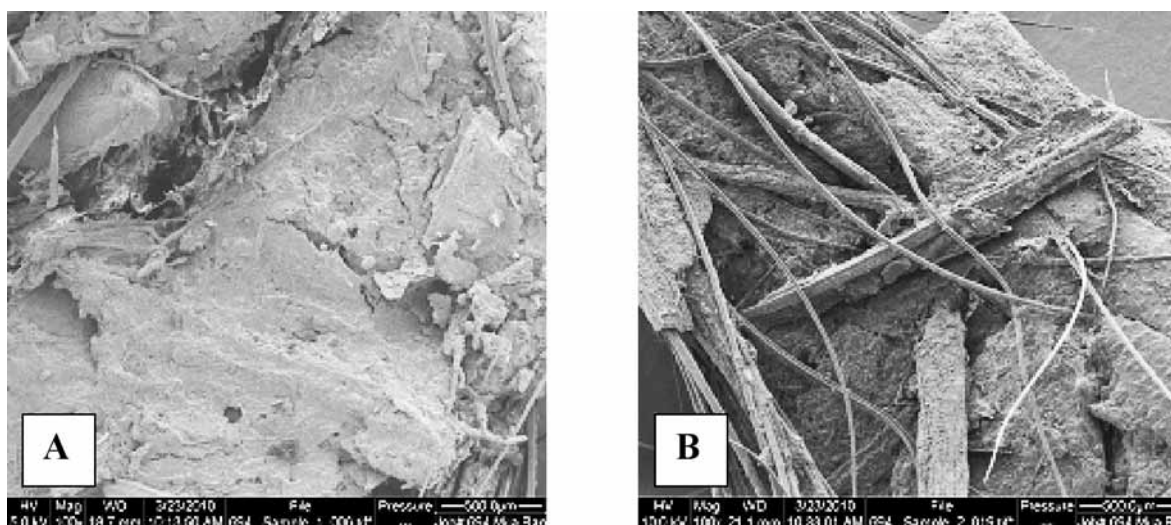


Figure 1. The SEM microscopic images of manure ball samples. Manure A is rich in clayish mud type (and softer when wet but hard when dry) while manure B has plenty of relatively larger undigested straw and fibers with fewer muddy portion than in A.

Each of the manure samples were inspected under the stereo- and scanning electron microscope and images were taken (Microscopic Images- ERRC core facility). By using the CT3-Texture Analyzer (Part No.CT350K115, from Brookfield Engineering, King of Prussia, PA) a trigger point of 10g was allowed to begin the test.¹⁷ This is how the CT3 detects when the probe is touching the sample. The relative work (in mJ) needed to cause a preset deformation at peak (def@peak) of 5 mm into each of the manure samples were measured before and after washing. The corresponding final load (in g) was also recorded for each sample. The quantitative softening of manure was noted, tabulated and compared. The relative amount of work, which corresponds to manure softening, was compared to the control without detergent.

RESULTS AND DISCUSSION

The manure samples that were collected and utilized were of similar physical texture, particularly a combination of the two types shown in Fig 1. When dried, manure A (with finer straw-like components than those found in B) had a tendency to become hardened like adobe hanging off the hair of the hides. Manure B had a tendency to disintegrate easier compared to A when wet. It could be due to the loose packing of the different components inside the manure balls. The relative efficiency of the different washing formulations on manure softening can only be compared if the amount of work can be measured quantitatively to cause a uniform amount of deformation in the manure sample before and after soaking.

TABLE I
Manure Sample Physical Properties and dimensions.

Sample (manure)	Volume (ml) (200% float)	weight* (g) (vacuum dried)	Sample dimension		Treatment formulation
			length (mm)	width (mm)	
1	6.8	3.4	32.9	10.6	pureglyc, pG
2	7.8	3.9	27.1	14.0	crudeglyc, cG
3	7.9	4.0	31.1	13.0	Na ₂ CO ₃ , SC
4	10.1	5.1	31.2	11.3	Nacarb+cG
5	10.5	5.3	32.9	12.3	SL+SC+cG
6	12.9	6.4	24.8	16.1	TnX114+sc+cG
7	11.8	5.9	36.0	12.2	Twn20+sc+cG
8	15.5	7.7	35.7	18.8	LDAO+SC+cG
9	13.2	6.6	33.7	13.0	SDS+SC+cG

TABLE II
CT3 texture Analyzer data of corresponding peak load and work needed for each manure sample taken at t=0, after 2 h and 4h soaking in different formulations.

Different	initially at t= 0	(t0)	after 2h soak	(t2h)	after 4h soak	(t4h)
Formulations	Fin Load, g	work, mJ	Fin Load, g	work, mJ	Fin Load, g	work, mJ
pure Glyc (pG)	2268	46	144	4.4	277	4.6
crudeGlyc (cG)	1805	63	260	7.0	254	7.7
(SC)	858	22	140	3.8	155	4.4
SC + cG	1308	39	78	2.7	259	3.7
SL + SC + cG	1325	37	206	6.7	132	3.6
TtnX114+SC+cG	1218	33	1021	25.0	593	15.3
Twn 20 +SC+cG	573	13	143	3.7	110	3.6
LDAO+SC+cG	558	25	205	5.8	93	3.3
SDS+SC+cG	817	22	388	10.3	369	10.5

Table I shows the physical properties and dimensions of the manure samples that were subjected to the corresponding differently prepared formulations listed in column 6. No two manures are alike thus, measuring the work needed at the beginning before the soaking/washing with the respective formulations and after 2 hours of soaking gave the relative work that was needed after soaking the manure. Overall, the work needed to cause the same deformation on the manure samples after soaking was much lower than before soaking but the rate of manure softening is different in the respective formulations as shown in Table II.

In Fig 2, it is shown that the initial work required to impinge 5 mm deformation into the nine different manure samples were never the same. The deformation at peak (def@peak)¹⁷ is

the sample deformation at the peak of the load. The trigger position (preset at 10g in this report) is the zero deformation reference point. Work is the area under the load curve and is measured in milliJoules. Final load (Fin load, g) is the load at the target deformation.

When the ratio of work after soaking over the work before soaking is taken and multiplied by 100 to get final percentage work for each manure sample, only then can the manure physical characteristics be normalized. The relative % work for each sample can then be compared quantitatively.

The comparison of the ratio of work needed from each sample washed in different formulation is shown in Fig 3. Soaking for 2 hours was relatively sufficient to soften the manure in almost

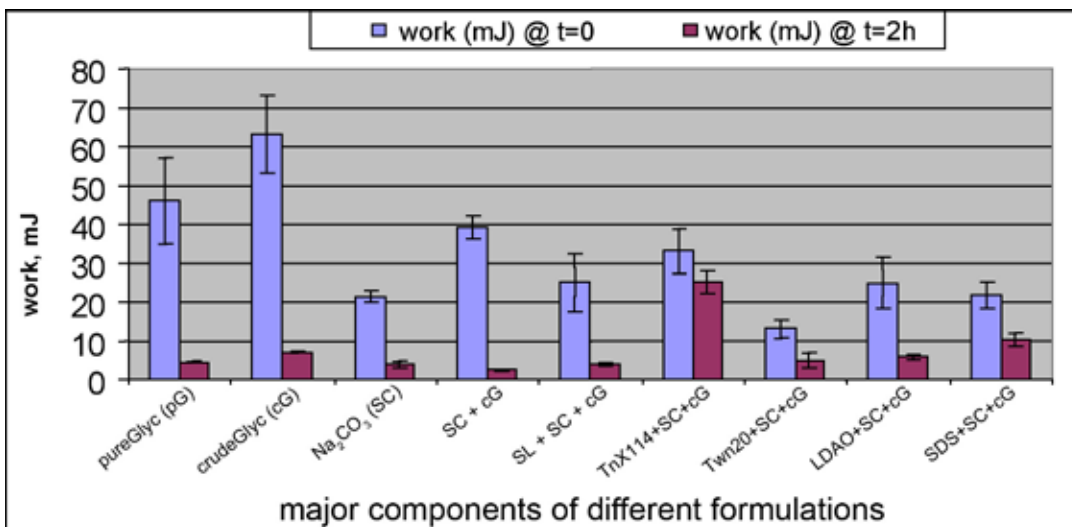


Figure 2. The amount of work (in mJ) needed to cause the same deformation at peak of 5mm into each manure sample at time 0 (t=0, blue bars) and after 2 hours washing (t=2h, maroon bars).

TABLE III
Comparison of % Work based on the ratio of work at t=2h/ t=0 compared to the ratio at t=4h/ t=0. Soaking for 2 hours was relatively sufficient to soften the manure in almost cases, except for Triton X-114 and LDAO.

Different formulations	work(t2h/t0)100	work(t4h/t0)100
pure Glyc (pG)	9.6	10.0
crudeGlyc (cG)	11.1	12.2
Sodium Carb (CS)	17.8	16.0
NaCarb+crdGlyc (SC+cG)	6.4	9.4
SophLipid +(SC+cG)	17.5	11.6
TritnX114 +(SC+cG)	75.8	46.4
Tween 20 +(SC+cG)	28.5	27.7
LDAO +(SC+cG)	23.5	13.4
SDS +(SC+cG)	47.5	45.4

all cases, except for Triton X-114 that incurred further softening after 4h soaking (from ~75 to 46%) and showed comparable degree of softening as the formulation with SDS at ~45% work.

The data shows that the manure softening is not as effective if the ratio of work after 2h over the work initially at t=0 is quite high. After 2 h, the manure washed or soaked in a formulation with Triton X-114 is still relatively the hardest with the highest ratio of ~70%. It is followed by the strong anionic detergent SDS with a ratio of ~ 46% then by the nonionic Tween 20 with

a ratio of ~35%. The softening is observed to be relatively efficient with the formulation containing the zwitterionic LDAO having a ratio of ~23% but the more efficient so far is with the nonionic SL, the biodegradable detergent with a ratio of ~15%. Among the 5 different surfactant used (all trials with the addition of 1% sodium carbonate and 10% crude glycerol), SL required the lowest amount of work or energy needed to deform the manure uniformly after soaking for 2 hours. The biodegradable surfactant, with the addition of recycled crude glycerol, was observed to be more effective in softening the hardened manure samples and will be further studied for “adobe” type manure removal.

Among the formulations without detergent, 10% crude glycerol alone works similarly as the 10% pure glycerol. The working mechanism of the surfactants may in part be based on its action as an amphiphile. The hydrophobic portion binds to the hydrophobic moieties of water insoluble compounds, rendering them hydrophilic inside its micelles and hence solubilized. The formulation made of 1% sodium carbonate and 10% crude glycerol is also promising. But its potential as a washing formulation can be assessed on its ability to eliminate the majority of microbial contamination in manures in future studies. A shorter contact time of 30 minutes or less will be explored and established so that the method will be more efficient and industry friendly. Different detergent types such as anionic (SDS), nonionic (Tween 20 and TritonX-114) and zwitterionic (LDAO) are represented and compared to the biodegradable surfactant, sophorolipid (SL). The biodegradable surfactant, with the addition of recycled crude glycerol, was observed to be more effective in softening the hardened manure samples and will be further studied for “adobe” type manure removal.

CONCLUSION

This study showed the relative efficiency of the different formulations on manure softening can be compared quantitatively by measuring the required amount of work to

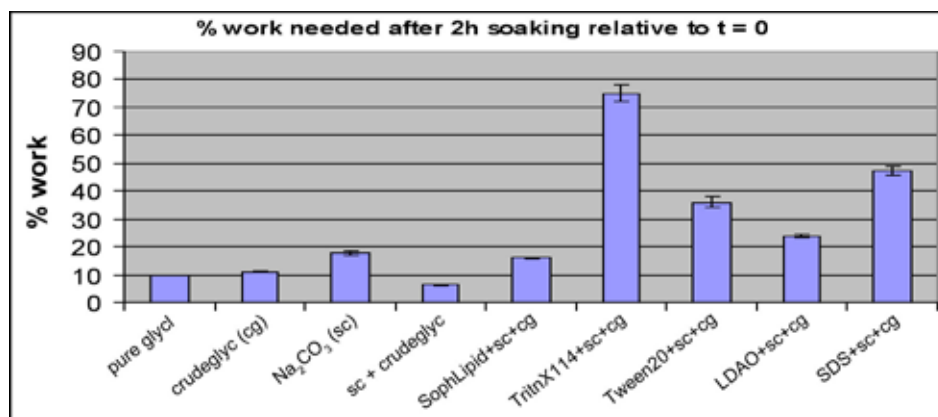


Figure 3. The relative % of work that is required on each manure after 2 h soaking with each respective formulation.

cause a uniform amount of deformation in each of the manure samples. When the ratio of work after soaking to the work before soaking was taken and multiplied by 100 to calculate the final percentage work for each manure sample, only then the manure physical characteristics can be normalized. The results showed that the crude glycerol is as efficient as the pure glycerol. It is a positive indication that this abundant co-product of biodiesel production can be utilized and recycled in removing hardened manure. The formulation made of just 1% sodium carbonate and 10% crude glycerol is also promising. But the potential of an effective soaking formulation is dependent upon its ability to eliminate the majority of microbial contamination in manures and this will be one of the subjects of future studies. Different detergent types such as anionic (SDS), nonionic (Tween 20 and TritonX-114) and zwitterionic (LDAO) are represented and compared to the biodegradable surfactant, sophorolipid (SL). The biodegradable surfactant, with the addition of recycled crude glycerol, was observed to be more effective in softening the hardened manure samples. It will be further studied for “adobe” type manure removal from its strong adherence to the bovine hide through the hair. The method can be improved further by exploring and establishing shorter contact time of 30 minutes or less (and by incorporating other cleaning agents) to gain practical applicability in the hide industry.

ACKNOWLEDGMENTS

The authors would like to thank the following: Joseph Lee, for his valuable help in procuring and handling of the hide samples from Dave Seaver and Bob Edmonston of JBS who arranged a tour in their facility at Souderton, PA; Susan Hogan of USHSLA for the valuable information and connections that led to the JBS raw hide processing plant visit; William N. Marmer (emeritus collaborator at ARS-ERRC), Gary Rennerfeldt (of Tyson Fresh Meats Inc.) and Ed Godsalve (member, Board of Directors of AMI-American Meat Institute) for the valuable discussions; and Doug Soroka of the Microscopic Imaging (MI) Core Facility at ERRC for the microscopic images.

REFERENCES

- Hogan, S. (2010). Report of US Hides, Skins and Leather Association (USHSLA) during the 2010 RLC meeting.
- Mitchell, J.W., Prevention of bacterial damage on brine cured and fresh cattle hides. *JALCA* **82**, 372-382, 1987
- Personal communications in the ALCA Research Liaison Committee (RLC) annual meetings, 2007-2010.
- Auer, N., Covington, A.D., Evans, C.S., Natt, M., Tozan, M. Enzymatic removal of dung from hides. *J. Soc. Leather Techn. Chem.* **83**, 215-219, 1999
- Covington, A. D., Evans, C.S. (2003) Enzymatic dung removal for improved tannery economics Proc. XXVII IULTCS Congress, Cancun, Mexico.
- Good glycerol information can also be found at this website; <http://www.pioneerthinking.com/glycerin.html>
- Aldema, M.L., McMurry, L.M., Walmsley, A.R. and Levy, S.B. Purification of the Tn10-specified tetracycline efflux antiporter TetA in native state as a polyhistidine fusion protein. *J. Mol Microbio.* **19**, 187-195, 1996
- Ashby, R.D., Nunez A., Solaiman D.K.Y et al. Sophorolipid biosynthesis from a biodiesel co-producer stream. *J. Am Oil Chem Soc* **82**, 625-630, 2005.
- Ashby, R.D., Solaiman, D.K.Y. Property control of Sophorolipids: influence of fatty acid substrate and blending. *Biotechnol Lett* **30**, 1093-1100, 2008.
- Mittal, K.L. and Fendler, E.J. eds., (1982) Solution Behavior of Surfactants: Theoretical and Applied Aspects, Plenum Press, New York, pp. 1261-1313.
- Linfield, W.M., ed (1973) Anionic Surfactants, M. Dekker, NY
- Tanford, C. (1980). The Hydrophobic Effect, 2nd ed. Wiley, NY
- Maeda, H. Dodecyldimethylamine oxide (DDAO or LDAO) micelles: stability, aggregation number and titration properties. *Colloids & Surfaces.* **109**, 263-271, 1996.
- Mager H, Rothlisberger R, Wagner F., Use of sophorolipid lactone for the treatment of dandruffs and body odor. European Patent EP 0209783, 1987.
- Pekin G, Vardar-Sukan F, Kosaric N., Production of sophorolipids from *Candida bombicola* ATCC 22214 using turkish corn oil and honey. *Eng Life Sci* **5**, 357-362. 2005
- Solaiman, D., Ashby, R.D. and Foglia, T.A. , Production of biosurfactants by fermentation of fats, oils and their coproducts. In: Hou, C.T., editor. Handbook of Industrial Biocatalysis. Boca Raton, FL: Taylor & Francis. **14**, 1-9. 2005
- CT3- Texture Analyzer Manual, Brookfield Engineering, Inc, Middleboro, MA. It can also be found at this website: <http://www.brookfieldengineering.com>