J. Dairy Sci. 101:1–10
https://doi.org/10.3168/jds.2017-13466

Adenosine triphosphate bioluminescence for hygiene testing of rubber liners and tubes on dairy farms

Ida Clemensson Lindell,* Åse Lundh,*[1] Kerstin Svennersten Sjaunja,† and Marika Cederholm‡
*Department of Molecular Sciences, Swedish University of Agricultural Sciences, PO Box 7015, SE-750 07 Uppsala, Sweden
†Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, PO Box 7024, SE-750 07 Uppsala, Sweden
‡DeLaval International, PO Box 39, SE-147 21 Tumba, Sweden

ABSTRACT

Prevention of biofilm formation in milking equipment is important to ensure good hygiene quality of raw milk. Key factors to achieving good results are a successful cleaning procedure and a method to check the cleanliness of milking equipment surfaces. Adenosine triphosphate bioluminescence is a fast and easy method for investigating bacterial contamination of surfaces. However, previous studies on the potential of ATP bioluminescence to assess the hygiene status of milking equipment have been hampered by lack of a validated test procedure. The aim of this work was therefore to establish a test procedure for assessing the cleanliness of milking equipment using ATP bioluminescence, and apply the method on-farm to study the hygiene status of aging rubber material in milking equipment. In developing the test procedure, the effects of sampling location in tubes and liners, sampling of dry versus wet barrels, milking point in the parlor, and acid or alkali detergent on ATP values were investigated. The results showed that, to obtain reproducible results, replicate sampling from the same milking points in the parlor is important. For milk tubes, samples should preferably be taken from the milk meter side, for liners on the inside of the barrel. For best results, sampling should be performed after use of alkali detergent. No beneficial effect was observed of sampling dry liner barrels, so sampling in the standardized test procedure is performed directly after cleaning. The standardized test procedure was used on 3 different commercial farms and sampling was initiated after replacement of old rubber parts. On one of the farms, additional sampling was performed to evaluate total bacteria count and determine the association with ATP level. The results suggest that, provided an efficient cleaning procedure is used, the hygiene quality of milking equipment can be maintained during the recommended lifetime of the rubberware. However, due to occasional variation in cleaning efficiency between milking points and liner barrels, random sampling on single occasions can lead to incorrect conclusions. Replicate sampling over time is therefore important for correct interpretation of ATP bioluminescence data. If ATP levels are very high, complementary sampling for total bacteria count should be used to verify that the level is due to bacterial contamination, and not other organic ATP-contributing material (e.g., milk residues).
Key words: milking equipment, ageing rubber material, standardized test procedure, field study

INTRODUCTION

Biofilm formation by bacteria in milking equipment is an important source of contamination of bulk tank milk (Elmoslemany et al., 2009; Verdier-Metz et al., 2009; Bava et al., 2011). To ensure good hygiene quality of raw milk, measures are required to prevent biofilm formation (Latorre et al., 2010). Factors contributing to biofilm formation in milking equipment include insufficient cleaning and hygiene, and aging of rubber material (Hillerton et al., 2004; Teixeira et al., 2005). As teat cup liners age, the chemical composition of the rubber material changes and therefore rubber liners usually have a recommended maximum life of 2,500 milkings. In studies by Hillerton et al. (2004) and Boast et al. (2008), it was found that liners aged for approximately 1,500 and 4,000 milkings, respectively, had an increased number of cracks and deposits of organic and inorganic material on the inner surface. Similar findings have been reported by Storgards et al. (1999), who studied aging rubber material in a dairy processing environment.

Efficient cleaning can reduce the risk of bacteria accumulating and forming a biofilm between milkings, and the clean-in-place (**CIP**) procedure is commonly used in different milking systems. Four major factors

Received July 10, 2017.
Accepted November 13, 2017.
[1]Corresponding author: ase.lundh@slu.se

contribute to the success of CIP in a milking system: (1) the thermal factor, determined by the temperature of the water; (2) the chemical factor, determined by the type and dose of detergent; (3) the time factor, determined by the length of the washing procedure; and (4) the physical factor, determined by the amount and turbulence of the water in the milking system (Reinemann et al., 2003; Teh et al., 2014). To ensure good hygiene quality of milk delivered to dairies, it is desirable to have reliable methods to assess the results of the on-farm cleaning procedure and the cleanliness of milking equipment surfaces.

Adenosine triphosphate bioluminescence has been shown to be a fast and easy method for investigating bacterial contamination of surfaces and assessing the efficiency of cleaning (Shama and Malik, 2013). The method is based on a reaction between ATP molecules and the enzyme luciferase with its substrate luciferin (Griffiths, 1993). In this reaction, ATP is converted to AMP with the emission of light at an intensity that is directly proportional to the amount of ATP in the sample (Shama and Malik, 2013). The method indirectly measures the number of microorganisms in a sample and results are obtained within minutes. The technique can be effectively used under field conditions but should be combined with microbiological testing because other nonbacterial organic material can also contribute ATP (Corbitt et al., 2000; Aycicek et al., 2006; Shama and Malik, 2013).

Previous studies using ATP bioluminescence to assess microbial contamination of milking equipment have been hampered by lack of a validated test procedure for measurements of ATP levels. Moreover, descriptions of the test procedures used in previous studies are incomplete, and large variations between measurements on individual farms and between farms have been reported (Slaghuis and Wiegersma, 1996; Reinemann and Ruegg, 2000; Vilar et al., 2008). The correlation between ATP level and total bacteria count (**TBC**) in milking equipment has also shown varying results in previous studies, from no correlation (Meyer and Schmidt, 1997) to a coefficient of determination (R^2) of 0.73 (Reinemann and Ruegg, 2000). Therefore, when using ATP bioluminescence, a reliable sampling procedure is needed that allows for correct interpretation of ATP readings and leads to better conclusions regarding the hygiene status of milking equipment.

The aims of this study were thus to (1) establish and evaluate a test procedure for hygiene testing of rubber liners and tubes using ATP bioluminescence and (2) apply the test procedure in the field to investigate deterioration of the hygiene status of aging rubber liners and tubes.

MATERIALS AND METHODS

Experimental Design

Three commercial dairy farms participated in the study that was conducted in 2 parts. In the method development part, ATP bioluminescence was used to establish and verify a test procedure for hygiene testing of rubber liners and tubes on the farm level. Criteria in developing the test procedure were that it could be used on farms without damaging of milking equipment, that the values obtained were representative of the general hygiene status of the equipment and that the method had high reproducibility. The test procedure was developed on one of the case farms (farm A) by investigating the effect on ATP level of 4 different parameters of relevance for field testing. Case farms and the different parameters are described further below.

In on-farm studies, the test procedure developed was then applied to investigate deterioration of the hygiene status of aging rubber liners and tubes. Newly installed milking equipment was sampled once a month on 3 farms, at intervals of 21 to 35 d. The variation in sampling interval was caused by practical circumstances on the farms. The manufacturers recommendation for using liners is up to 2,500 milkings or 6 mo, whatever comes first (DeLaval, Tumba. Sweden). Because sampling continued for 6 mo on all farms in the study, the liners were used for at most 4,050 milkings. In addition, the correlation between ATP readings and TBC was evaluated on one farm.

Establishment of Test Procedure

The 4 parameters investigated in development of the test procedure were (1) sampling location on the inside of liner and tube, (2) sampling of dry versus wet liner barrels, (3) milking point in the parlor, and (4) type of detergent. One parameter at a time was varied while the others were kept constant. The effect of sampling location on liners was tested by swabbing 3 different surface areas inside the liner: the lip (approximately 16.5 cm^2), the head (approximately 35 cm^2), and the barrel (approximately 80 cm^2). On milk tubes, swabs were taken inside the tube at both ends (approximately 2–15 cm into the tube, 60 cm^2). To investigate the effect of sampling dry versus wet liners, all liners of a cluster were dried for 4 h before swabbing. Drying was performed by removing liners from the jetter after cleaning and allowing them to air dry in a vertical position with the lip facing downward. The resulting ATP readings were then compared with those taken immediately after the cleaning procedure. The effect of milking point location