LWT - Food Science and Technology 90 (2018) 505–512

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Microbial load of white cheese process lines after CIP and COP: A case study in Turkey

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ARTICLE INFO

Keywords:
Dairy
Process line
Biofilm
ATP-Bioluminescence
Food safety

ABSTRACT

Microbial adhesion, contamination and biofilm formation are major problems in the dairy industry, in cheese production, and for consumer health. In our study, the microbial loads of white cheese process lines were examined after cleaning treatments. *Bacillus* spp. was determined as the most common species on process lines. Drainage, air, and water entry points were found as important contamination points. Biofilm forming capacity of *Enterobacteriaceae* as revealed using Congo Red agar, was very high. In addition, the standard counting and ATP-Bioluminescence methods were applied and compared for evaluating biofilms. The ATP-Bioluminescence method was found useful for rapidly identifying the biofilm-formable surfaces.

1. Introduction

Milk and dairy products are very nutritive products that have been significant for human life since ancient times. In Turkey, the dairy industry is a big part in the economy, and nearly 70% of Turkish dairy production belongs to white cheese production (Temelli, Anar, Sen, & Akyuva, 2005). Small manufacturers generally produce cheese by using rennet enzyme without adding a starter culture.

Biofilm can easily form on dairy process lines and surfaces due to the available nutrients and humidity. *Lactobacillus* spp., *Streptococcus* spp., *Lactococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Shigella* spp., *Citrobacter* spp., *Flavobacterium* spp., *Klebsiella* spp., *Proteus* spp, *Enterobacteriaceae*, *Listeria* spp, *Pseudomonas* spp, *Bacillus* spp., *Debaryomyces* spp. and *Saccharomyces* spp. were isolated from dairy process lines and process surfaces like doors, walls, drains and floors (Sharma & Anand, 2002; Temelli et al., 2005; Gündüz & Tuncel, 2005; Martins, Pinto, Rocha, De Araujo, & Vanetti, 2006; Cherif-Antar et al., 2016; Schön et al., 2016). Food poisoning microorganisms can be a part of biofilm microbiota due to cross-contamination and, they can form biofilm by themselves which is responsible for cross-contamination. Moreover, biofilms can harm surfaces and damage instruments and equipment by promoting corrosion; thus resulting in energy and economic losses, and increasing the treatment costs (Poulsen, 1999).

Clean in Place (CIP) and Clean Out Place (COP) are two procedures used in the dairy industry. NaOH, HCl, and HNO_3 are used for removing organic and inorganic dirt. Water is used for pre-cleaning and removing chemical residuals. Brushes are used as mechanical cleaners. Disinfectants (chlorine or peracetic acid etc.) and vapor are used for sanitation. CIP procedures are used for milk tanks, brine tanks, pasteurization units, and pipelines. COP procedures include water rinse, disinfectants, vapor and UV like treatments. It is generally used for knives, curd cutting knives, cheesecloth, cheese vats, walls and floors etc. (Dufour, Simmonds, & Bremer, 2004).

In Ezine region, Çanakkale, Turkey, an irreversible bad-odor problem was observed in cheese products. Firms examined their raw materials and the bad-odor problem was not reported from raw materials. So, it was decided to check out the process lines. In this study, microbial loads of Ezine cheese process lines, the efficiency of cleaning procedures to remove this microbiota and availability of using rapid methods like ATP- Bioluminescence for microbial load examination were investigated. Another aim of this study is to determine the biofilm forming and/or bad odor producible organisms in the dairy microbiota since biofilm status of a food plant is an important pre-requisite for effective HACCP implementation and sanitation procedures should be modified to eradicate the biofilm.

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https://doi.org/10.1016/j.lwt.2017.12.062
Received 3 April 2017; Received in revised form 17 November 2017; Accepted 26 December 2017
Available online 02 January 2018
0023-6438/ © 2017 Published by Elsevier Ltd.

2. Materials and methods

2.1. Sample collection

In our study, samples were taken from three SME's dairies. They have nearly 70 personnel and 60–80 ton capacity milk/day. Additionally, they are cheese exporters in Çanakkale region, Turkey.

Sampling points[1] were defined with dairy technicians. Samples were collected in three different seasons (April to November 2015) after cleaning. First sampling was done in April 2015 at average temperature 12.5 °C for Çanakkale. The second sampling was done in July 2015 at average 25 °C. Third sampling was done in November 2015 at average temperature 11.8 °C. However, production area temperatures were stable at 30 °C; because of cheese production. Filling rooms' temperatures were between 15 and 20 °C. For determining the cleaning efficiency, samples were collected both before and after CIP/COP from one dairy's process area. All samples were analyzed within 4 h of collection.

2.2. Method

2.2.1. Surface and air sampling

Surface swab samples were taken from the equipment and personal hands. Sampling was done with standard swab method and ATP-bioluminescence methods. In standard method, 100 cm^2 areas were swabbed with a swab pre-wetted in 0.1% sterile peptone water. Samples from the stainless steel and plastic pipe surfaces were taken after opening available connection parts. Personnel swab samples were taken from both hands. In ATP-bioluminescence method, ATP content was measured in relative light units (RLU) by using a luminometer (ATP-Luminometer PD-10 Kikkoman Co., Japan.). The cleanliness of the surfaces was assessed according to their microbial load (İpek & Demirel Zorba, 2014).

Air samples were taken from production and filling area of dairies. Air sampler instrument (SKC Biostage- 28,3 L/min) was used during 4 min.

2.2.2. Enumeration of microbial loads and isolation

Surface samples from the production lines were assayed by the aerobic mesophilic bacteria, *Pseudomonas*, *Bacillus*, *Listeria* spp., *Enterobacteriaceae* counts. Aerobic Mesophilic Bacteria (AMB) loads were determined by Nutrient Agar (NA, Merck 1.05450) at 35 ± 2 °C/24-48 h, *Enterobacteriaceae* counts were determined by Violet Red Bile Glucose Agar (VRBGA, Merck 110275) at 37 °C/24-48 h, *Pseudomonas* spp. counts were determined by CFC Selective Agar with its supplement (CFC, Merck 1.07620 + Merck 107627) at 30 °C/24–48 h, *Bacillus* spp. were determined by Hichrome *Bacillus* agar (Hichrome M1651) at 30 °C/24–48 h and *Listeria* spp. counts were determined by PALCAM *Listeria* Agar with its supplement (PALCAM, Merck 1.11755 + Merck 112122) at 37 °C/24–48 h} (AOAC, 2000; Hitchins, Jinneman, & Chen, 2016; Tewari, Singh, Singh, & Kumar, 2012; İpek & Demirel Zorba, 2014). Isolates were taken from all media and examined for cell morphology and Gram reaction. Then they were stored in nutrient agar slants at 4 °C and also in Nutrient Broth with 15% glycerol at −18 °C. Isolates were identified to genus level by using basic phenotypical methods such as oxidase, catalase, motility, glucose usage with/without O_2, growth on MacConkey Agar (Merck, 105465), Voges-Proskauer, methyl red, carbohydrate fermentation and haemolyses tests etc (Cullimore, 2000). Then isolates were identified with bioMerieux API kits.

[1] Water, raw material-milk tanks, balance tank, stainless steel pipes, plastic pipes, knives, curd cutting knives, cheesecloth, cheese vats, walls and flours, plastic cups of rennet enzyme, air sample of production and filling areas, hands of personnel working in production and filling areas, brine tank.

2.2.3. Biofilm capacities of isolates

Biofilm formation capacities were determined by using Congo Red Agar. After 37 °C/24 h incubation, black colonies were accepted as biofilm positive (Kala, Chauhan, Rajput, & Kutty, 2012).

2.3. Statistical analyses

Analysis of variance was performed in order to compare surface microbial loads values -which were obtained by standard counting methods and surface RLU values -which were obtained with ATP-bioluminescence methods by statistical software SPSS (IBM SPSS Version 22 for Windows, IBM SPSS Inc., USA). The correlation was measured to determine the strength and direction of the relationship between different surface sample counts using the standard and ATP-BM methods.

3. Results and discussion

3.1. The cleaning and disinfection protocols of dairies

The cleaning and disinfection routines in each firm are summarized in Table 1. In all dairies, Clean in Place (CIP) and clean out of place (COP) procedures were applied as cleaning treatments. They use 2% NaOH for caustic rinse and 1.5% HNO_3 for acid rinse. The temperatures and the application periods of caustic, acid and water rinse in the CIP procedures varied between the dairies.

3.2. Microbial loads of process line

Aerobic mesophilic bacteria (AMB), *Enterobacteriaceae*, *Pseudomonas* and *Bacillus* loads of samples were given in Table 2 and Fig. 1. Microbial loads of surfaces, air and water samples were between < 1.00 and 6.84 log CFU. In Dairy A, Aerobic mesophilic bacteria, *Enterobacteriaceae*, *Pseudomonas* and *Bacillus* spp. loads of the surfaces, air and water samples decreased during 2nd and 3rd samplings. This dairy made an improved arrangement in the process area by changing process equipment arrangement and pipes between 2nd and 3rd sampling period, according to our suggestions. This improvement and lower environmental temperatures resulted in a decrease in microbial loads. In Dairy B process lines, A big alteration was done in process area just before our 2nd sampling. So, a slight increase in microbial loads was determined between 2nd and 3rd sampling period. *Bacillus* spp. was observed as the common microorganism on all surfaces. They were isolated from all surfaces in Dairy A and B and 78.5% of surfaces in Dairy C.

Sharma and Anand (2002) and Cherif-Antar et al. (2016) investigated that *Bacillus* spp. is a common bacteria in dairy process lines. In researches about the effect of disinfectants on spore-forming bacteria, it was reported that chlorine and the peracetic acid application has lower activity on this type bacteria (Gopal et al., 2015; Ostrov, Harel, Bernstein, Steinberg, & Shemesh, 2016). Two dairies that we take samples from, use chlorine but the other uses peracetic acid for disinfection. This might be one of the reasons of *Bacillus* spp determination. Likewise, groundwater usage in hygiene and sanitation procedures, and the location of production building could be other reasons for *Bacillus* spp. contamination. It is a significant contamination risk for the process area. Furthermore; walls, drainage, and brine tanks were determined as the key contamination points for this dairy process area. Drainages and walls are important, because of the humidity in the process area. Drainage sourced microorganisms can contaminate the products by vaporization. Walls' microorganisms can contaminate products by vaporization and drips.

Listeria spp. were determined in the various areas mainly non-food contact surfaces cleaned by COP procedures. In dairy A's process line, *Listeria* spp. was isolated from water, stainless steel pipes, cheese vat, wall, drainage and brine tank (1.0–1.97 log CFU/100 cm^2). In dairy B process line, *Listeria* spp. was isolated from water, stainless steel pipes, plastic pipes, cheese vat, production and filling personnel hands, wall,

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