ASSESSMENT AND CONTROL OF BIOFILM FORMATION IN DAIRY PROCESSING SYSTEM

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（牛乳加工工程におけるバイオフィルムの評価と制御）

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論 文 内 容 の 要 旨

It is known that dairy-related microbial biofilms can cause continuous contamination leading to poor keeping quality, lowered shelf-life, nutritional deficiency and milk-borne diseases. Besides, they are able to create serious problems namely mechanical blockages, impedance of heat exchangers, and bio-corrosion of equipment materials. Therefore, the objective of this study is to assess and control biofilm formation in dairy processing system. The control of bacterial biofilms was focused on two methods: control of an environmental condition, milk pH during long-term biofilm formation in skim milk; and inhibition of initial attachment of bacteria on stainless steel surface after a conditioning process.

For the former method, milk pH was controlled at neutral values during 14 days of long-term biofilm formation of dairy-related bacteria on stainless steel coupons in different dilutions of skim milk. During incubation at 30°C, in pH-adjusted samples, milk pH was daily kept at around 7.0 by addition of sterile sodium hydroxide, while in pH-unadjusted samples, milk pH spontaneously decreased due to bacterial development. Biofilms were stained by 0.1% Crystal Violet solution and daily assessed by optical density method. Bacterial counts of suspensions showed that the adjustment of pH enhanced the growth of bacteria in free-floating form. In contrast, optical densities of biofilms in the pH-adjusted samples were significantly lower than in the pH-unadjusted samples. The study also found that thicker biofilms were formed in milk dilutions at higher nutrient levels. These results mean that control of milk pH and nutrient level could significantly reduce biofilm formation of the tested bacteria.

For the latter method, to control initial step of bacterial attachment, surface conditioning with skim milk solutions (5%) at pH from 3 to 10 was investigated. Little milk deposit was found on surface conditioned with alkaline milk, while significant milk foulants were made on stainless steel coupons conditioned with acidic milk. The conditioned surfaces were then used to assess bacterial adherence. The enumeration of adherent cells under a fluorescence microscope implicated that surfaces which were previously conditioned with acidic milk had the lowest adherence among tested surfaces. Bacterial attachment was also increased with the bacterial exposure time from 30 min to 3h and 12 h. The results suggested that in a short contact time (30 min and 3 h), surface conditioning with acidic milk could reduce bacterial adherence. However, this effect did not last for longer bacterial exposure time (12h).

The utilization of dairy by-products for surface conditioning was also proposed due to their economical significance and their low risk of chemical pollution to main products. The study showed that, under examination of a confocal laser scanning microscope, the conditioning layers formed by skim milk, buttermilk and butter serum were the thinnest, medium and the thickest, respectively. Assessment of bacterial adherence on treated surfaces showed that, in the majority of cases, the adherence-reducing abilities of buttermilk and butter serum were better than skim milk. While skim milk could reduce bacterial

adherence during shorter exposure time (30 min), buttermilk and butter serum could act during longer period (up to 12h). Therefore, in order to decrease bacterial adherence for short bacterial exposure period (30 min), surface conditioning with all tested dairy by-products is recommended. For longer bacterial exposure period (3h and 12h), only surface conditioning with buttermilk and with butter serum could be advisable.