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Fouling of Milk on Heat Transfer Surface with and without the Addition of Bacillus stearothermophilus – A Laboratory Study

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Fouling of Milk on Heat Transfer Surface with and without the Addition of Bacillus stearothermophilus – A Laboratory Study

Jinah Yoo, Xiao Dong Chen, and Bipan Bansal

Abstract

Fouling of heat transfer surfaces during milk processing is a major problem for dairy industry. In the current work, the nature and extent of milk fouling with and without the addition of Bacillus stearothermophilus in the feed solutions have been studied. A fouling detection system based on electric conductivity measurements has been used. The coresponding thermal resistance of the deposit layer has also been worked out. The fouling deposits have been analysed visually as well as with a scanning electron microscope (SEM). The results show significant variation in the fouling patterns with and without the large presence of bacteria in the flowing medium.

KEYWORDS: Bacillus stearothermophilus, milk fouling, bio-fouling, dairy processing

1. INTRODUCTION

Biological fouling is the accumulation of biologically derived material onto processing surfaces. Micro-organisms can adhere to a heat transfer surface or an established fouling layer or they can get entrapped inside the fouling matrix (Changani et al, 1997). Once the micro-organisms are deposited, an established fouling layer which is full of nutrients would enable them to grow and multiply. The most common type of micro-organism present in milk fouling is Bacillus stearothermophilus (Murphy et al, 1999). These micro-organisms will deteriorate the quality of milk when they are released into process streams during processing from the established colonies or biofilms (or bio-fouling patches). Fouling material dislodged by fluid hydrodynamic shear can also contaminate milk (Visser et al, 1997).

During heat treatment of milk, the whey protein molecules start to denature and aggregate, some of them attach themselves onto the process surface to form the fouling layer at temperatures greater than 60-70°C. Also the solubility of calcium sulphate decreases with increasing temperature. Therefore, it is expected to precipitate and contribute to the thickening of the fouling layer. The presence of micro-organisms can also contribute to the build-up of the fouling layer (biofilm alone or mixture layer with milk components).

Two types of milk deposits normally occur during its processing: type A and type B (Changani et al, 1997). The characteristics of these deposits are affected by different factors like milk composition, temperature and pH, temperature difference between the heating surface and milk, and the characteristics of the surface (e.g. roughness) where the deposits form. The composition of these deposits depends on the temperature at which milk is processed and also the process surface temperature. Type A deposits occur between 70°C and 110°C and type B deposits occur over 110°C. Type A deposits are white, voluminous and contain 50-60 % proteins, 30-50 % minerals, and 4-8 % fat (Visser et al, 1997). Type B deposits are hard, granular, and grey in colour and consist of 70-80 % minerals, 15-20 % proteins, and 4-8 % fat (Visser et al, 1997). According to Belmar-Beiny and Fryer (1995) the initial layer of deposits consists mainly of protein and this happens irrespective of the processing temperature.

The aim of this study was to investigate the effect of the deliberate addition of Bacillus stearothermophilus on the milk fouling rate and the deposit structure. The operating conditions were maintained to ensure only type A fouling, the most common type of fouling in the dairy processing plants (except the UHT process).

2. MATERIALS AND METHODS

Micro-organism

Bacillus stearothermophilus was used in this study as it is one of the main thermophilic contaminants of milk. The strain used was isolated from a dairy factory which makes milk powder by researchers at the Microbe Research Centre, Waikato University, New Zealand. The sample bacteria were stored at -20°C in a sterile 50% v/v solution of Glycerol and Trypticase Soy Broth. Inoculants for the experiments

were grown up from these stored cultures. Inoculation of the test media (the milk solution) was initially to a concentration of 10^2 cfu ml^{-1}.

The Fouling Rig

The fouling rig is shown in Figure 1. It is mainly made up of milk tank (A), cleaning solution tank (B), tank heaters (inside of A and B), test section (C), reference section (D), control equipment (E), and recording equipment (F). A data logging system is used to record heater temperature (T_s), milk temperatures at the inlet (T_{in}) and the outlet (T_{out}) of the test section, and milk temperature in the heated zone (T_{bulk}).

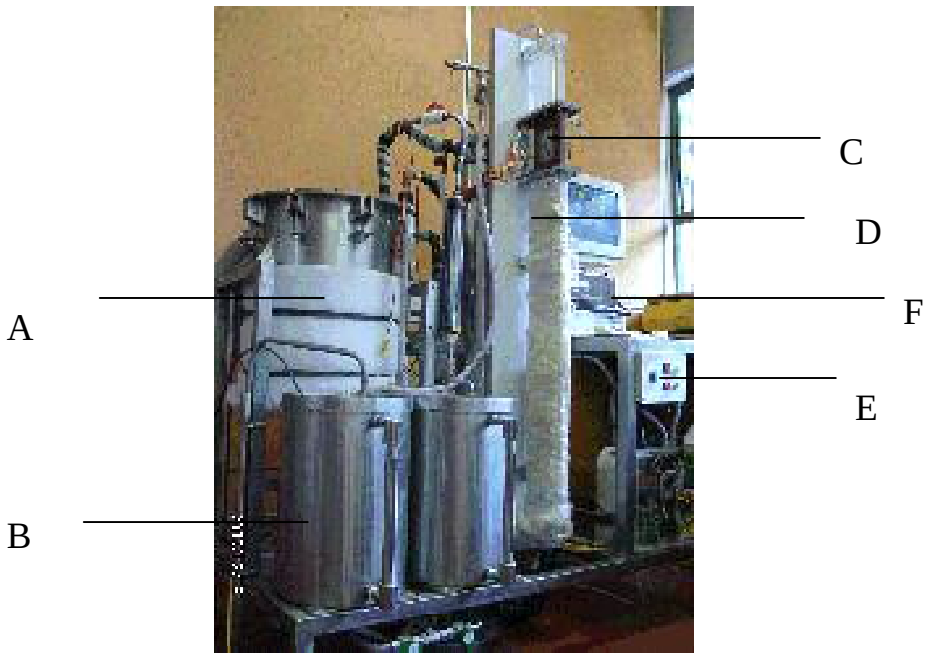

Figure 1: The photo of fouling apparatus: milk tank (A), cleaning solution tank (B), fouling test section (C), reference section (D), control equipment (E) and data recording equipment (F).

20 litres of 10 wt % milk was reconstituted from skim milk powder (NZ Milk Products). The milk was heated in the tank to 60°C for about 30 min and then pumped through the rig at 0.05 ms^{-1}. The milk was recycled during the experiments. The temperature of the heating surface of test section was maintained at 85°C, 90°C or 95°C. As a result, milk got heated at the wall and fouling took place. This process was carried out for either 3.3 or 7 hours. In order to ensure fully developed laminar flow conditions, milk flowed for 1.44 m before entering the test section.

The rig was sanitized after each run using water (≈ 20 min), 3 wt % sodium hydroxide solution (≈ 1 hour), 2 wt % nitric acid solution (≈ 1hour) and finally water

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rinsing (≈ 20 min). This helped to dissolve the fouling material and minimise bacterial contamination between runs.

The Data Acquisition

Data were acquired by LABTECH data acquisition system (Laboratory Technologies Corporation (c), USA- 1994). Measurements were taken every 30 seconds using a computer attached to the data acquisition system. The computer contained 3 boards: a Keithley Das-1601 board, an Exp-16 board and a SAT-08 board.

The related calculations

Figure 2 shows a schematic diagram of the fouling rig. The details of the fouling test section are also given. Two identical sets of electrodes were used, with one set for the reference section and one set for the test section. These electrode sets, placed opposite to each other inside the pipe, measured the electrical resistance of milk in the reference section (R_{test}) and sum of the electrical resistances of milk and deposit layer in the test section (R_{ref}). The resistance of the deposit layer was determined by subtracting the R_{ref} value from the R_{test} value.

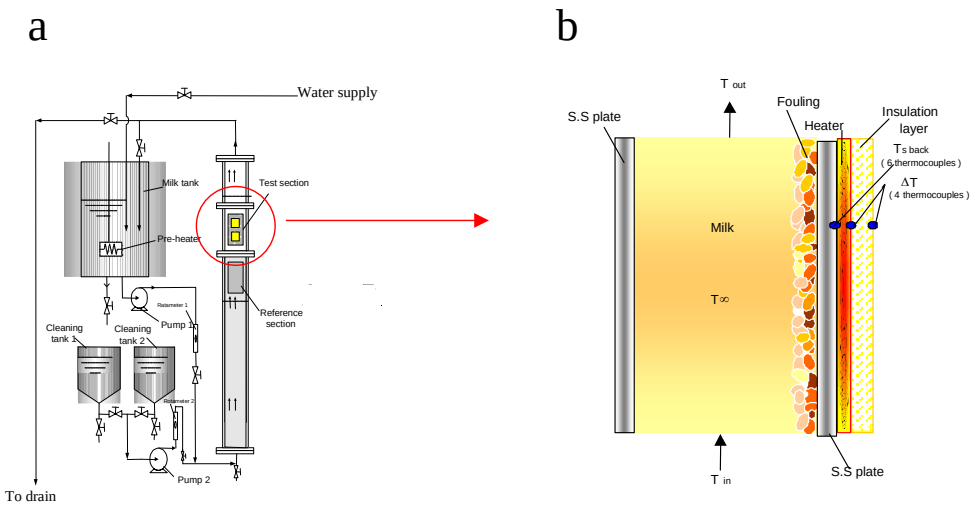

Figure 2: (a) A schematic diagram of fouling apparatus, (b) Test cell for calculating the thermal resistance.

As illustrated in Figure 2, maximum surface contact was ensured between the heater and the heated surface to minimize the heat loss. The temperature at the back of the heating surface was measured by six K type thermocouples. The heater was insulated by a 10mm thick insulation. The heat loss was calculated by measuring the temperature difference between both sides of the insulation using K

type thermocouples. These temperatures and the heater power were used to calculate the fouling thermal resistance (Chen et al, 2003)

Visualisation of Deposit Layer

The heating surface in the test section was made of stainless steel 304L. Another stainless steel surface with five viewing windows was located on the opposite side to allow the visual inspection as illustrated in Figure 3. Visual inspection of the deposit layer at different surface temperatures (85°C, 90°C, or 95°C) was done using a digital camera (Pentax Digital Still Camera, Asahi, OPT. Co. Ltd.).

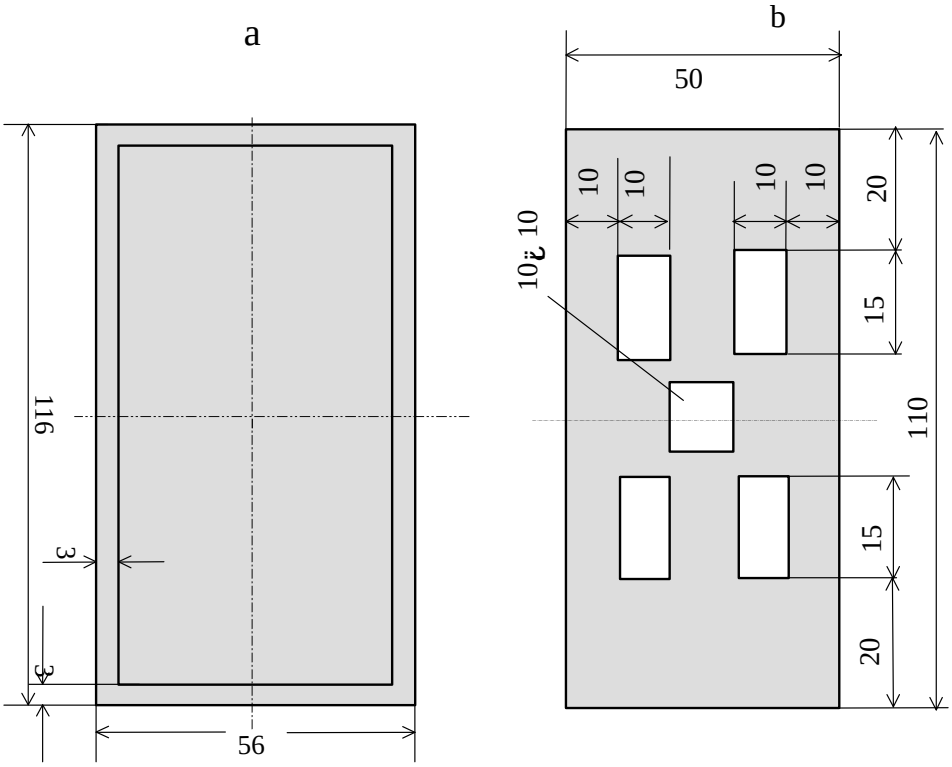

Figure 3: Schematic diagram of fouling test section: (a) fouling surface of stainless steel 304L, (b) stainless steel with 5 viewing windows on opposite of the fouling surface (all dimensions are in mm).

Thickness

At the end of a fouling experiment, the fouling surface was rinsed with water at 25°C for 1 minute to eliminate the materials, which were only loosely attached to the fouling surface. Then the thickness of the deposit layer was measured using a calliper as shown in Figure 4.

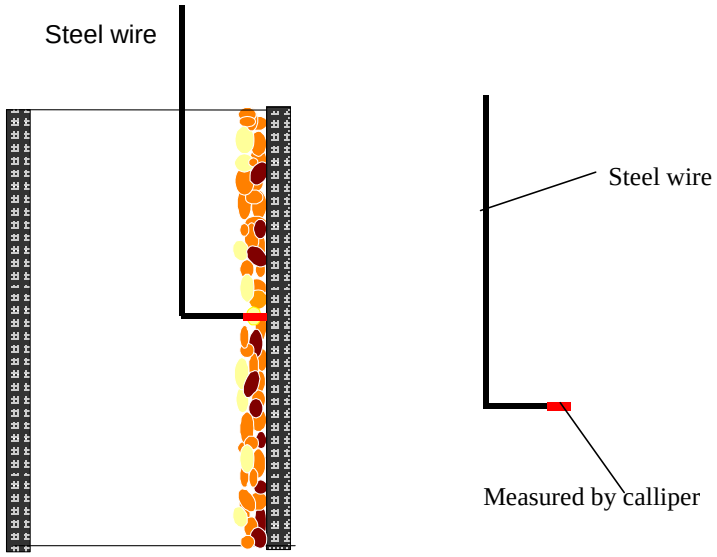

Figure 4: Measurement of fouling layer thickness by calliper.

Scanning Electron Microscopy (SEM) Analysis

SEM (Philips XL30 S-FEG, Holland) operating at 5 kV was used to characterise the structure of the deposits formed at deposit/solution interface and heating surface/deposit interface. Before examination in the SEM, the samples were coated with platinum in a Polaron Sputter Coater (SC7640 VG Microtech, England).

3. RESULTS AND DISCUSSION

Milk Fouling without and with the Addition of Bacillus stearothermophilus

Recently, a novel deposit gauging technique employing electrical signal has been developed and tested in our laboratory (Li, 2002 and Chen et al, 2003). It was found that the electrical resistance increased with increasing the thermal resistance of the deposit layer. In the current study, the milk fouling generated with and without the addition of Bacillus stearothermophilus was studied by measuring both electrical and thermal resistances.

According to Li (2002), the temperature and milk concentration have an impact on the milk fouling. Two different sets of experiments were performed in the current study:

- Set I - First set of experiments were performed using reconstituted skim milk without the addition of Bacillus stearothermophilus.
- Set II – Second set of experiments were performed using reconstituted skim milk with the addition of Bacillus stearothermophilus.

In both cases, the reconstituted skim milk solutions were pasteurised by heating to 90°C and then holding at that temperature for 1 minute. Then 10^2 cfu/ml of Bacillus stearothermophilus was added in milk during the second set of experiments. It is worth mentioning that no Bacillus stearothermophilus growth was observed through out the processing period during the first set of experiments.

Milk fouling without the addition of Bacillus stearothermophilus

Three different fouling experiments were performed (heating surface temperature 85°C, 90°C, and 95°C). No Bacillus stearothermophilus was added in milk during these experiments.

Figures 5 and 6 show the electrical resistance and thermal resistance respectively measured during the experiments. It can be seen that fouling took place in a typical pattern. A distinctive period was observed at the start of the fouling process where no fouling took place. This is induction period and is illustrated by almost little changes in both resistances. This is in line with the findings of Fryer (1997). This induction period may be a result of the time it required to develop suitable conditions for fouling. Figures 5b and 6b show the logarithmic plots to highlight the induction period. Longer induction period was observed at the lower wall temperature as expected.

Extensive fouling started after the induction period. With the heating of milk, the reactive sulphydryl groups of the protein molecules get exposed (denaturation). They react with each other and form protein aggregates. These aggregates then attach to the heat transfer surface and cause fouling. Higher fouling was observed with the increasing wall temperature.

Figure 7 shows the fouling curves (using electrical resistance) observed during the experiments that were 7 hours long. In all the experiments, the fouling level settled to asymptotic values after around 5 hours of operation. The resulting electrical resistance values at the end were greater than 6Ω.

As mentioned earlier, a calliper (see Figure 4) was used to measure the thickness of the fouling layer at the end of the experiments. Tables 1 and 2 show the thickness values for the experiments, which were 3.3 and 7 hours long respectively. All the experiments were repeated twice to get reproducible results.

As observed with the electrical resistance and thermal resistance curves (see Figures 5 and 6) fouling was enhanced when the heater surface temperature was higher. The effect of temperature was much more evident in these early stages of the fouling process. The fouling resistance curves settled to similar asymptotic values near the end (see Figure 7). Hence similar values of deposit thickness were observed after 7 hours of operation.

Milk fouling with the addition of Bacillus stearothermophilus

There are various ways in which the presence of Bacillus stearothermophilus in milk can influence the fouling process:

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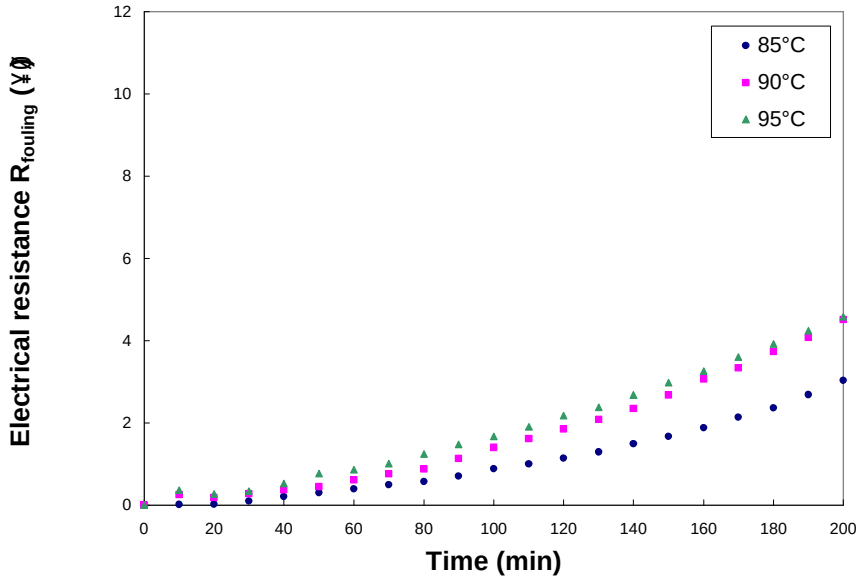

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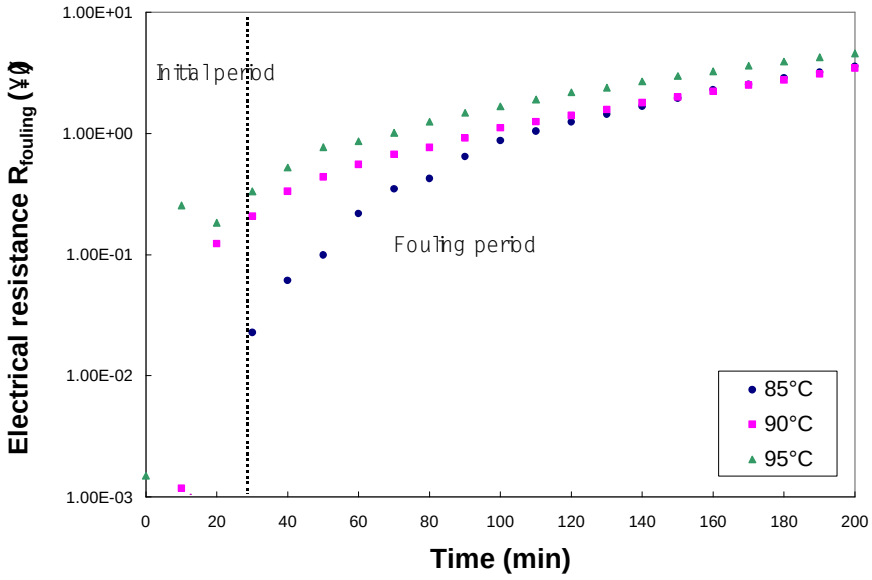

Figure 5: Milk fouling without the addition of Bacillus stearothermophilus for 3.3 hours using electrical resistance: (a) general overview, (b) logarithmic view to highlight the initial period.

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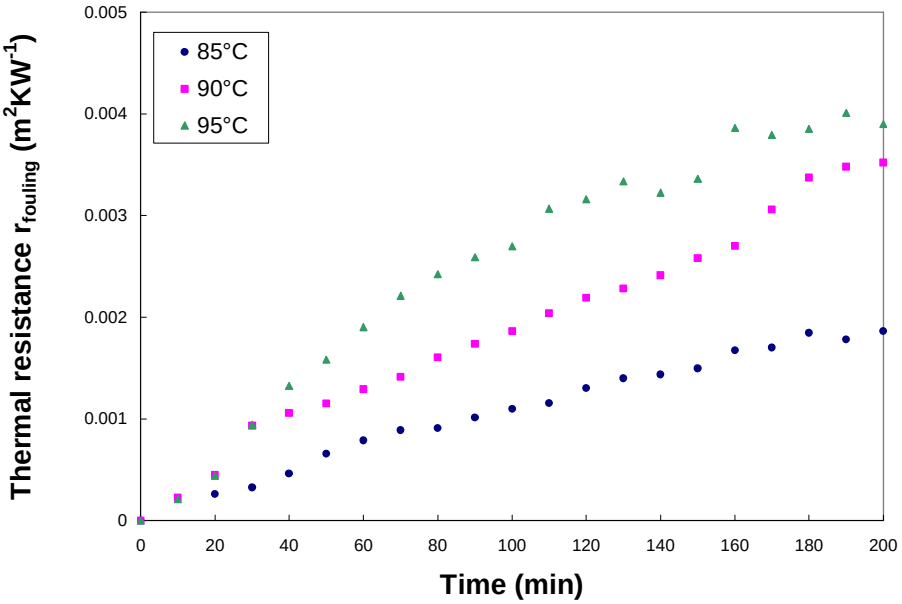

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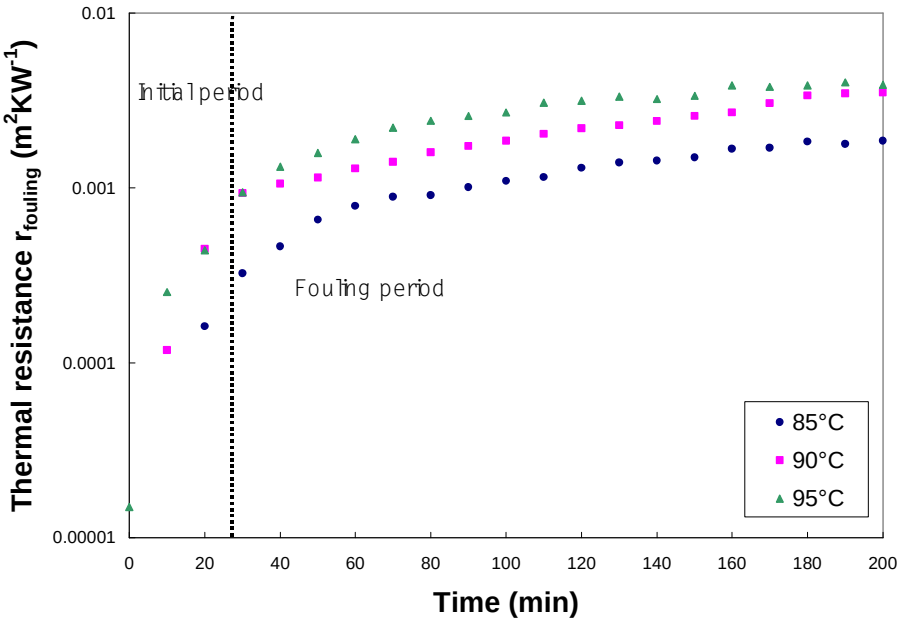

Figure 6: Milk fouling without the addition of Bacillus stearothermophilus for 3.3 hours using thermal resistance: (a) general overview, (b) logarithmic view to highlight the initial period.

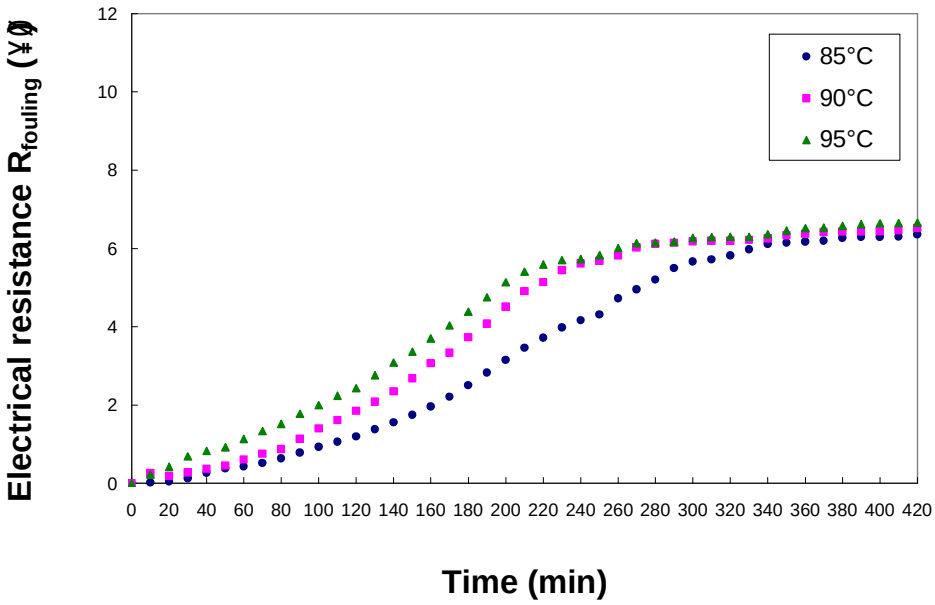

Figure 7: Milk fouling without the addition of Bacillus stearothermophilus for 7 hours using electrical resistance.

Table 1: The thickness of the fouling deposit layer without the addition of Bacillus stearothermophilus at 3.3 hours.

Experiment	Thickness (mm)			
		Repeat I	Repeat II	Average
85°C	1.2	1.2	1.2	1.2
90°C	1. 9	1.7	1.8	1.8
95°C	2.0	2.0	2.0	2.0

Table 2: The thickness of the fouling deposit layer without the addition of Bacillus stearothermophilus at 7 hours.

Experiment	Thickness (mm)			
		Repeat I	Repeat II	Average
85°C	3.2	3.2	3.1	3.2
90°C	3.3	3.4	3.2	3.3
95°C	3.2	3.3	3.4	3.3

- When Bacillus stearothermophilus, an acid-producing bacteria, is allowed to grow, the milk pH falls (Yoo et al, 2004). This results in the formation of milk protein clusters, which settle on the heat transfer surface.
- Bacillus stearothermophilus produces protease as mentioned in our previous paper (Yoo et al, 2004). This is a protein splitting enzyme, which breaks down the milk proteins into smaller protein fragments, either peptide or amino acid.
- Bacillus stearothermophilus also produces glycocalix, which consists of polysaccharide chain. This chain protrudes from the cell wall and has end groups, which enable the bacteria to stick to various surfaces (Dudridge et al, 1983 and Costerton et al, 1983).
- Bacillus stearothermophilus itself deposits on the heat transfer surface, which may aid further fouling.

The first three mechanisms would take some time to be effective.

Three different experiments were carried out (heating surface temperature 85°C, 90°C and 95°C) to study the influence of the addition of Bacillus stearothermophilus (10^2 cfu ml^{-1} in the feed tank) on the milk fouling. Apart from the addition of the bacteria all operating conditions were kept same as in the previous set of experiments performed without the bacteria.

Figure 8 shows that the fouling pattern changed significantly with the addition of Bacillus stearothermophilus. Minimal fouling was observed for up to 6.4 hours at 85°C (electrical resistance ≈ 0.5Ω). Similar results were observed at 90°C and 95°C. In comparison, the electrical resistance values were significantly higher without the bacteria (≈ 6Ω, see Figure 7).

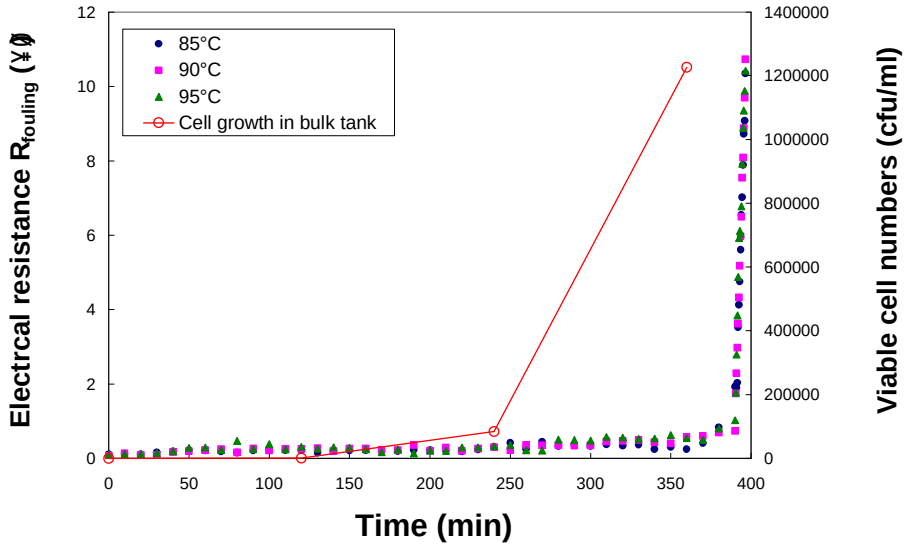

Figure 8: Growth of Bacillus stearothemophilus in bulk tank and milk fouling with the addition of Bacillus stearothemophilus for 7 hours using electrical resistance.

The possible explanation for the mitigation of fouling by the bacteria is that the overall deposit strength reduced in the presence of Bacillus stearothermophilus and as a result the deposits were removed more easily by the hydrodynamic forces exerted by the process stream. The deposit removal process was confirmed by visual observations.

The concentration of the bacteria in the feed tank increased continuously from the initial value of 10^2 cfu/ml through out the fouling process. At 6 hours of operation, the concentration in the feed tank was measured to be 1.2×10^6 cfu/ml as shown in Figure 8. This was a result of the nutrients supplied by in the feed liquid. Interestingly, the electrical resistance of fouling did not changed much until the bacteria concentration in the feed tank was reached to 1.2×10^6 cfu/ml. The previous experience suggested that the bacteria concentration would increase more considerably after that (to the order of 10^7 cfu/ml) (Yoo et al, 2004). At such high concentrations, significant biologically induced fouling took place. The enhancement in the fouling rate was so significant after 6.4 hours of operation that the electrical resistance increased from 0.5 Ω to 10 Ω within around 0.5 hours. After the experiment, the deposit thickness was measured to be over 8 mm thick, which was about 2.5 times thicker than the deposit layer observed without the addition of Bacillus stearothermophilus (see Table 3).

Table 3: The thickness of the fouling deposit layer with the addition of Bacillus stearothermophilus at 7 hours.

Experiment	Thickness (mm)			
		Repeat I	Repeat II	Average
85°C	8.1	8.1	8.3	8.2
90°C	8.1	8.2	8.2	8.1
95°C	8.2	8.1	8.2	8.2

Visualisation of the Fouling Process

Visual inspection of the deposit formed on the heating surface was made under the following conditions:

- Fouling process without the addition of Bacillus stearothermophilus
  - 3.3 hours long experiments
  - 7 hours long experiments
- Fouling process with the addition of Bacillus stearothermophilus
  - 3.3 hours long experiments
  - 7 hours long experiments

Fouling generated without the addition of Bacillus stearothermophilus

Figures 9, 10, and 11 show the photographs of the deposits formed without the addition of Bacillus stearothermophilus. The deposits were porous, soft, and

creamy white in colour. Figures 9b, 10b, and 11b show the close-ups and clearly indicate that with the increase in temperature, the amount of deposits increased. Also the deposits were comparatively less porous and had more compact structure at the higher temperature.

Further visual observations were made after 7 hours of operation and the deposit photographs are shown in figure 9c, 10c, and 11c. The fouling mass increased with the processing time. Also most of the porous structure was filled by the deposits and the resulting surface was smoother. The brownish colour of the deposits may be due to Maillard reaction.

Fouling generated with the addition of Bacillus stearothermophilus

As mentioned earlier, the milk fouling was minimised with the addition of Bacillus stearothermophilus for up to 6.4 hours of operation. During this period, a few deposit clusters were observed as shown in Figure 12a and 12b. The bacteria may form clusters with milk aggregates, which then get attached to the stainless steel reversibly by electrostatic attraction. These clusters were not strongly attached with the heating surface and were prone to removal by the hydrodynamic forces.

After 7 hours of operation significant changes were observed in the deposit layer (Figure 12c). The deposits were thicker and more proteinaceous compared with the deposits obtained without the addition of Bacillus stearothermophilus (see Figure 9c).

Micro-structure Study using SEM

The SEM study was carried out to investigate the effect of the addition of Bacillus stearothermophilus on the morphology of the fouling deposits. For the SEM analysis, the samples were prepared by cutting small portions of the deposits immediately after the experiments and then freeze drying them using a bench-top 3.3/Vacu-freeze dryer. The samples were taken from two different sets of deposits:

- Deposit formed at deposit/solution interface
  - without the addition of Bacillus stearothermophilus
  - with the addition of Bacillus stearothermophilus
- Deposit formed at heating surface/deposit interface
  - without the addition of Bacillus stearothermophilus
  - with the addition of Bacillus stearothermophilus

Deposit formed at deposit/solution interface

The SEM micrographs in Figure 13 show the surface structure of the milk fouling generated without the addition of Bacillus stearothermophilus after 3.3 hours of operation. At 85°C, the deposits seemed to be highly porous and were merely set (Figure 13a). At 90°C, the deposits were more compact with a lower porosity (Figure 13b). At 95°C, the deposits were dense (Figure 13c).

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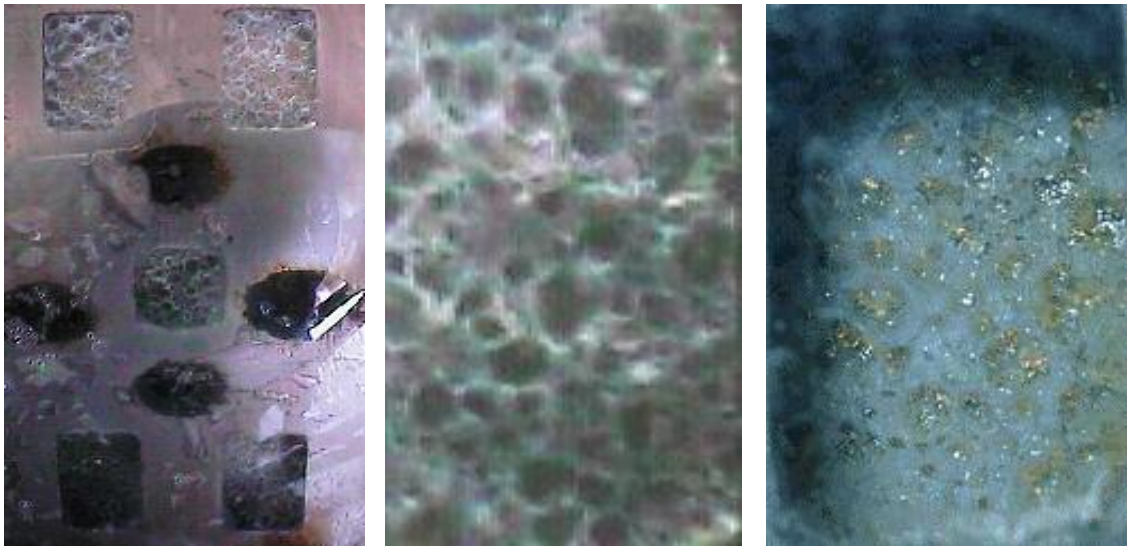

Figure 9: Photographs of the fouling deposits formed at the surface temperature 85°C without the addition of Bacillus stearothemophilus: (a) at 3.3 hours, (b) close up of (a), (c) close up at 7 hours.

a b c

Figure 10: Photographs of the fouling deposits formed at the surface temperature 90°C without the addition of Bacillus stearothemophilus: (a) at 3.3 hours, (b) close up of (a), (c) close up at 7 hours.

a b c

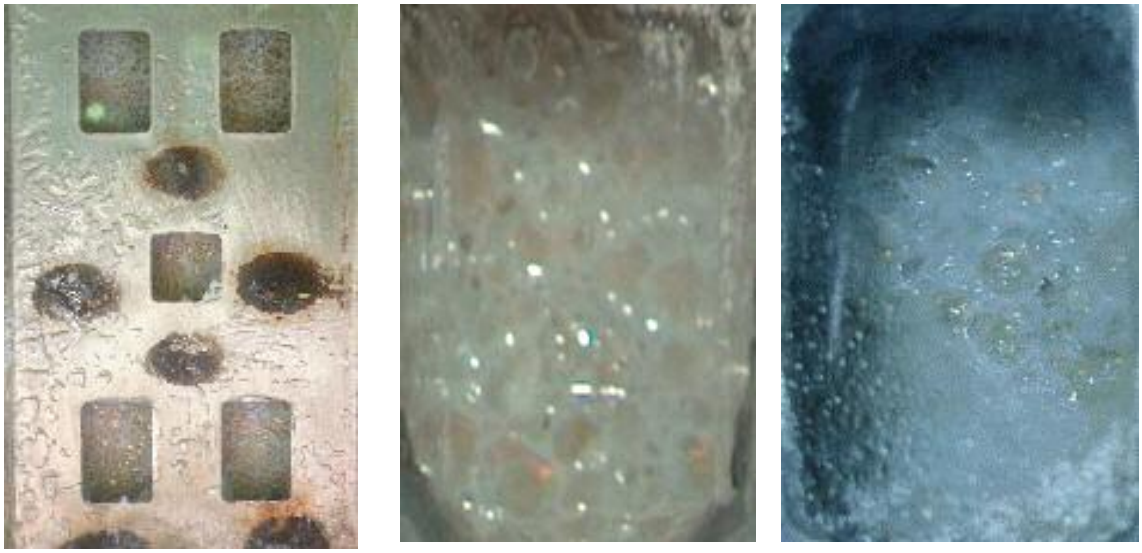

Figure 11: Photographs of the fouling deposits formed at the surface temperature 95°C without the addition of Bacillus stearothemophilus: (a) at 3.3 hours, (b) close up of (a), (c) close up at 7 hours.

a b c

Figure 12: Photographs of the fouling deposits formed at the surface temperature 85°C with the addition of Bacillus stearothemophilus: (a) at 3.3 hours, (b) close up of (a), (c) close up at 7 hours.

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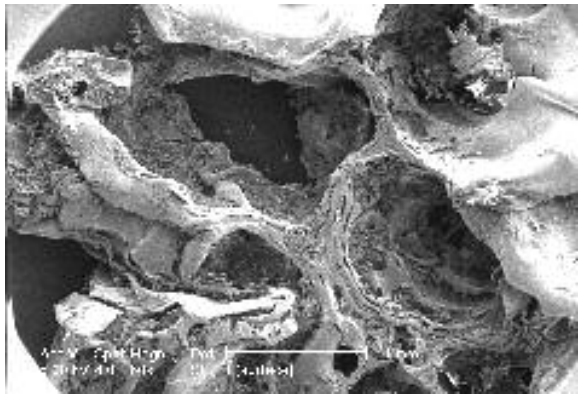

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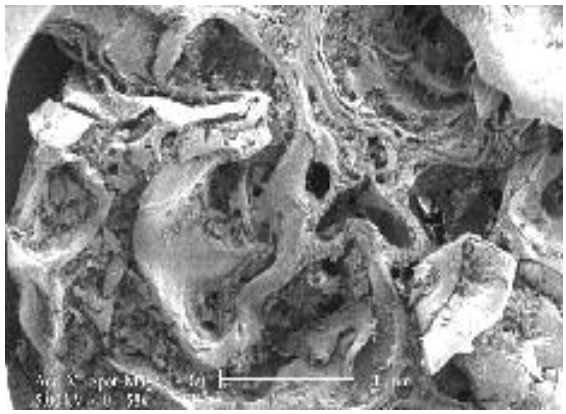

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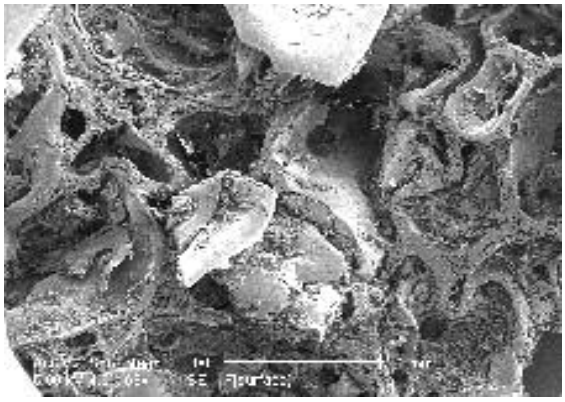

Figure 13: SEM micrographs of the deposits formed at deposit/solution interface at 3.3 hours (without the addition of Bacillus stearothermophilus): (a) at 85°C (mag. x 60), (b) at 90°C (mag. x 65), (c) at 95°C (mag. x 66).

After 7 hours, the heating surface was almost covered by the protein aggregates and the resulting deposits were very thick and had a smoother appearance. This is clearly evident in Figure 14 showing the deposits formed at 85°C. Similar results were obtained at the higher temperatures.

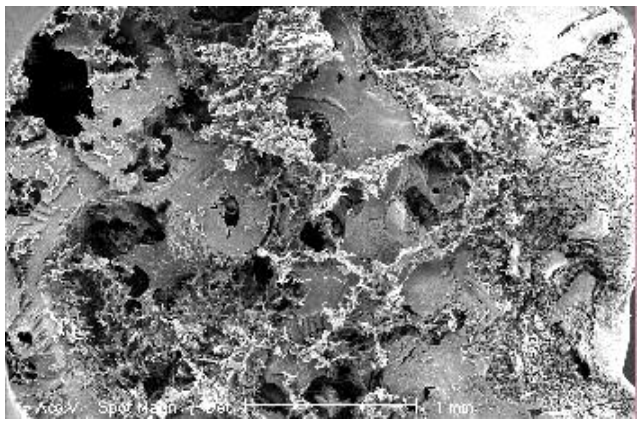

Figure 14: SEMmicrograph of the deposits formed at deposit/solution interface at 85°C at 7 hours without the addition of Bacillus stearothermophilus (mag. x 67).

As explained earlier, the addition of Bacillus stearothermophilus (10^2 cfu/ml) prevented the milk fouling for up to 6.4 hours at all the temperatures. Only after that period, when the Bacillus stearothermophilus concentration increased considerably, fouling was able to take place. Figure 15 shows the structure of the deposits formed after 7 hours of operation. The micrograph in Figure 15 clearly shows that the deposits were less compact and more voluminous compared with the deposits formed without the addition of Bacillus stearothermophilus (see Figure 14).

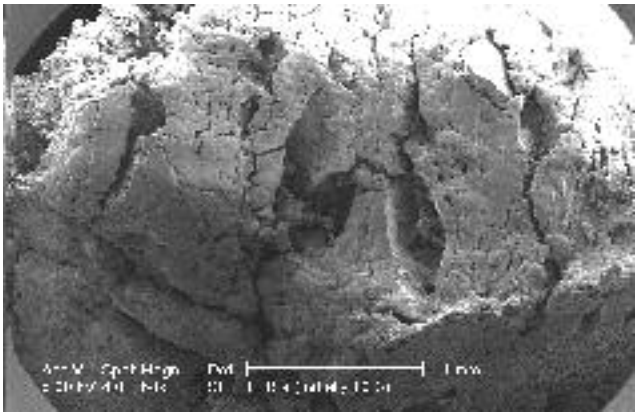

Figure 15: SEMmicrographs of the deposits formed at deposit/solution interface at 85°C at 7 hours with the addition of Bacillus stearothermophilus (mag x 68).

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Deposit formed at heating surface/deposit interface

Figure 16a shows the structure of the deposits formed on the heating plate without the addition of Bacillus stearothermophilus. As observed with the deposits formed at the deposit/solution interface, the deposits became more compact with the increasing temperature. Figure 16b shows a close up of the deposits.

Figure 17a shows less porous and more compact deposit structure compared with the one without the addition of Bacillus stearothermophilus. Figure 17b shows a high magnification micrograph of a large protein cluster settled on the fouling matrix. A fibrous and hairy structure can be observed. These observations show the "bridge" linkages as well as the presence of the bacteria in the deposit layer.

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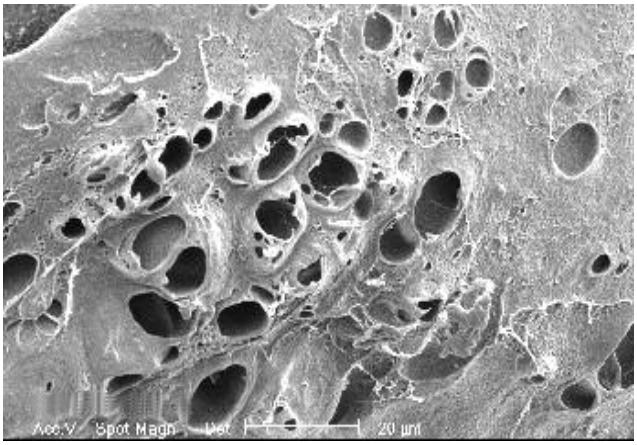


b

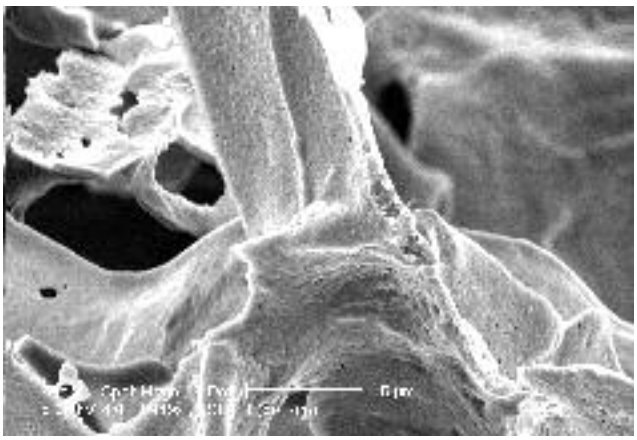
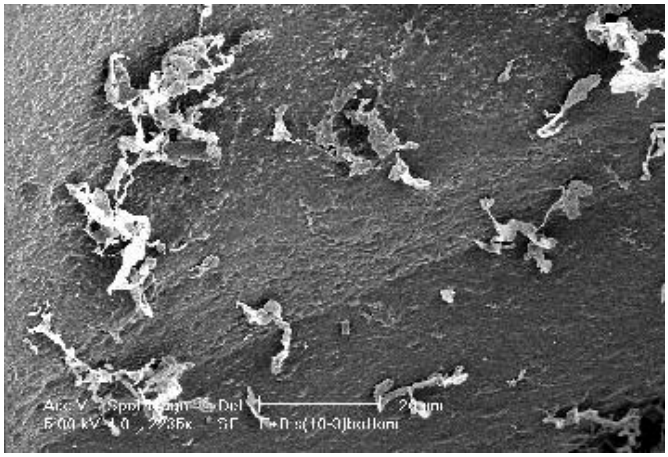


Figure 16: (a) SEM micrograph of the deposits formed at heating surface/deposit interface at 85°C at 7 hours without the addition of Bacillus stearothermophilus (mag. x 2137), (b) a close up (mag x 8949).

a



b

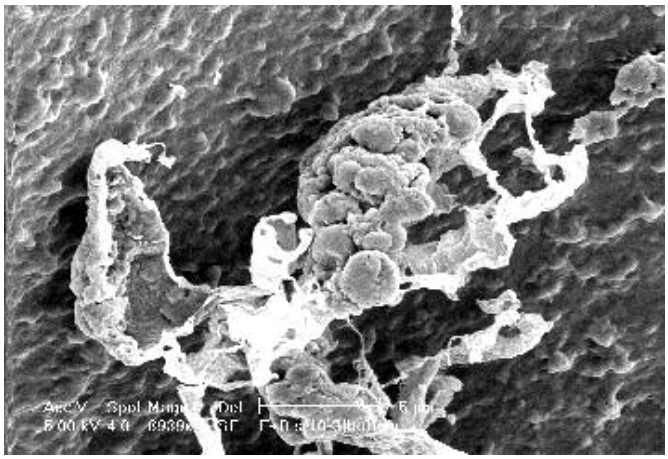


Figure 17: (a) SEM micrograph of the deposits formed at heating surface/deposit interface at 85°C at 7 hours with the addition of Bacillus stearothermophilus (mag x 2235) , (b) a close up (mag x 8939).

4. CONCLUSIONS

Milk fouling experiments were performed without and with the addition of Bacillus stearothermophilus in the feed solutions at three different temperatures. The fouling progress was monitored by measuring the electrical resistance and thermal resistance of the deposit layer. The deposits were generally porous, voluminous, and creamy white in colour. With the addition of Bacillus stearothermophilus the fouling process changes significantly. Fouling was found to be not significant for up to 6.4 hours of operation. The overall strength of the

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deposits was apparently reduced by the bacteria indicating an effect of the products from bacteria growth. However, beyond 6.4 hours of operation, the concentration of the bacteria in the tank increased considerably and as a result significant fouling took place. Ultimately, one can see that if the milk is contaminated with the bacteria over time, one should expect massive fouling. The cleaning of the fouling of this kind has also been studied and will be discussed in a future paper.

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