Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 17(66) No. 1 – 2024
https://doi.org/10.31926/but.fwiafe.2024.17.66.1.7

IMPROVING QUALITY CONTROL MEASURES IN THE DAIRY INDUSTRY: UTILIZING DYNAMICS OF DIRT RESISTANCE FACTOR (R_d) VALUE AND TIME OF EFFECTIVE BACTERIAL CONTAMINATION PREVENTION

Budianto BUDIANTO[1] Wahyu T. UTOMO[1]
Anung SAPUTRO[1] Annisa W. INDRIANI[1]

Abstract: *The number of customer complaints related to decreased quality of dairy products was the background of this study.* ***Objective****: (i) examined the effect of the highest dirt resistance factor (R_d) on the number of bacterial contaminants in finished goods; (ii) examine the effect of time on the growth of bacterial contaminants in the fouling layer of UHT pipes when not in operation.* ***The research method*** *was based on the mathematical derivation of the formation process of Rd in the form of an equation. Bacterial species were analyzed using the Operation Taxonomic Unit (OTU) with 16S rRNA. The ANOVA test was used to determine the mean value, standard deviation and the Tukey LSD post hoc test to see the difference in heat balance and bacterial growth.* ***Findings****: (i) During the UHT sterilization process, the Rd value (0.0205 $h.Ft^2.F.Btu^{-1}$) had an impact on total bacterial contamination >20 $Cfu.mL^{-1}$; (ii) In the event of engine damage or malfunction of the heater on the UHT. Time had a significant effect ($p<0.05$) on the number of lactic acid bacteria in the fouling layer in UHT pipes.* ***Conclusion****: The study recommends a sanitation schedule every 110 minutes (± 2 hours) or Rd value around 0.0205 $h.Ft^2.F.Btu^{-1}$.*

Key words: *Cleaning In Place (CIP), fouling, milk, Ultra High Temperature (UHT).*

1. Introduction

Dairy products undergo a unique transformation to achieve the quality required by existing food safety regulations. Therefore milk producers always try to find the best processing line to provide optimal sanitary and economic conditions. The number of customer complaints about the quality of milk in

[1] Chemical Engineering, Al-Kamal Institute of Science and Technology, Jakarta, Indonesia.
Correspondence: Budianto Budianto; email: budianto_delta@yahoo.com.

processed dairy products that go stale quickly became the basis of this research. Most of the processed dairy companies in Indonesia have complained about this. These conditions must take into account the physical and biochemical modifications of the raw materials during the production process [16].

Many previous studies agree that the process of protein denaturation and mineral deposition play a key role in fouling in the sterilization area [7, 16, 20].

However, the rate of formation of fouling and contaminant microorganisms will differ depending on the composition of the milk and the process [11]. Fouling is formed not only based on mass balance flow, heat balance and mechanical properties during the process [5]. There is also no agreement regarding the relationship between mass balance and heat balance which is a factor in the formation of fouling on the growth of microorganisms. This study wanted to see the relationship of these variables to microorganism contaminants. This has become a unique and attractive strategy for dairy companies in quality control.

Fouling is a major problem in the thermal performance of equipment that affects production costs [11]. The formation of fouling in the sterilization area causes a decrease in product quality due to the failure of the process fluid to reach the required temperature [16]. A serious problem with soiling is that surface cleaning is expensive [5, 11].

The main focus of this research is to control the Critical Control Point (CCP) area against microorganism contamination during the processing. The high nutritional value of milk causes milk to become a medium that is very favored by microorganisms [7] so that in a very short time milk becomes damaged if not handled properly [25]. The sterilization area has an important role in controlling microbial contamination. Sterilization with Ultra High Temperature (UHT) encourages the formation of a mineral layer faster thereby reducing the effectiveness of heat transfer from the heating medium to dairy products. This is closely related to the sterilization process which is the CCP area in the processing of these dairy products [23].

This study used a Double Pipe Heat exchanger; the mechanism of heat transfer occurs indirectly [17]. The fluid with a lower temperature (cooling fluid) flows through a small pipe while the fluid with a higher temperature flows in a larger pipe (annular pipe). The heat transfer that occurs in the fluid is a convection process [8] while the conduction process occurs in the pipe wall. This heat exchanger will not be effective in the sterilization process if there are a lot of impurity deposits [10], which are influenced by fluid composition, fluid temperature, tube wall temperature, and fluid flow velocity [6].

This study is empirical and aims to: (i) examine the effect of the highest Rd on the number of bacterial contaminants in finished goods; (ii) examine the effect of time on the growth of bacterial contaminants in the fouling layer of UHT pipes when not in operation. Both objectives were used as a reference for the sanitization schedule in the form of cleaning in place (CIP). The large amount of mineral deposits on the heat exchanger pipes carried by the fluid flow is suspected to be a source of microorganism contamination.

CIP is an attempt to clean the remaining milk from the equipment without

disassembling it, in this case the UHT heat exchanger [12, 14]. This research is very important as an effort to find sanitation standards based on the formation of fouling (product flow composition, flow velocity, heat exchanger temperature) against microorganism contamination

2. Materials and Methods

2.1. The Sample Used

The study sample was pure cow's milk from cattle farmers in West Java, Indonesia. The characteristics of the milk are shown in Table 1.

Sample characteristics Table 1

No	Sample	Batch	Milk quality in buffer tank			
			Fat [%]	Protein [%]	Total Solid [%]	Density [kg/l]
1	Milk	1904001	2.17	2.21	17.01	1.0589
2		1904002	2.20	2.24	17.02	1.0592
3		1904003	2.15	2.19	17.00	1.0591
4		1904004	2.10	2.18	16.98	1.0584
5		1904005	2.13	2.19	16.99	1.0588

Note: The analysis was done using Milcoscan FT-2. Sample characteristics include: % fat, % protein; % total solid and density which gives results that are almost the same or not much different from other batches.

2.2. Work Procedures

This study was based on standard operating procedures at a dairy processing company in Jakarta, Indonesia: a) milk samples for the UHT process were first tested for quality; b) during the UHT process, the temperature was recorded on the sensor every five minutes; c) microbiological test, sampling of the fouling layer in the sterilization pipe (UHT area), and after the UHT area by swab method after the sterilization process stops; d) Microbiological testing is divided into two, namely (i) the final product after the UHT process, and (**ii**) microbiological tests on the distribution pipes of the UHT area when Rd is around 0.0205. In this test, the pipes are removed, and then tested for the number of bacteria based on time (every hour).

2.3. Technique of Data Analysis

Determination of the Resistance Dirt Factor (R_d) value for the five batches includes a) heat balance; b) Log Mean Temperature difference (LMTD) and ΔT value; c) heat temperature (T_c); d) Reynolds number in the inner pipe and annulus; e) heat transfer factor; f) heat transfer coefficient; g) determination of fluid viscosity ratio; h) corrected heat transfer coefficient; i) net overall heat transfer (U_c); j) gross overall heat transfer (U_d).

2.4. Process Flow Chart

The processing of dairy products is carried out in the company as the object of research. The results of the data and observations show the existing processes so far. Data collection is assisted by instrument sensors as shown in Figure 1

and Table 2.

Table 2 describes the location and function of the instrument sensors and the materials used in the inner pipe and annulus. The design specifications of the tool include: pipe inner diameter, pipe thickness, flow area and fluid type. Mathematical equations refer to Angeletti and Moresi [1] and Budianto et al. [4]. The calculation begins with the mass balance and the heat balance between the two streams, namely the shell (hot) and the tube (cold). The mass flow can be seen in the following Figure 2.

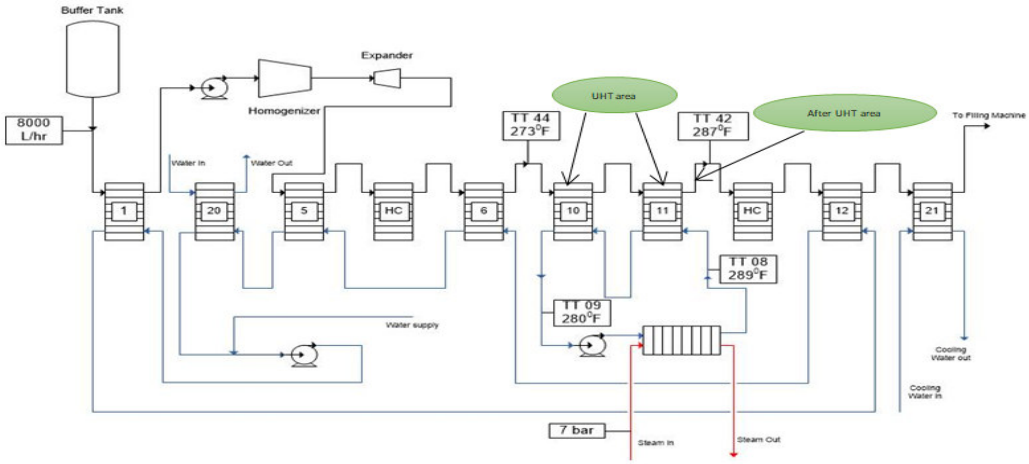


Fig. 1. *UHT process flow diagram*

Sensor instrument Table 2

No	Sensor	Location	Description
1	TT-44	Before entering the sterilizing section UHT	Sensor inlet temperature of the product
2	TT-42	After exit sterilizing section UHT	Temperature sensor exit product
3	TT-08	Before entering the sterilizing section UHT	Hot water inlet temperature sensor
4	TT-09	After exiting the UHT sterilization section	Temperature sensor comes out of hot water
5	PT-44	Before entering the UHT sterilization section	Steam inlet pressure on the PHE

Tool Design			
Inner pipe		Anullus	
Pipe Inner Diameter	0.115 ft	Pipe Inner Diameter	0.1725 ft
Pipe Thickness	0.0256 ft	Pipe Thickness	0.0354 ft
Flow Area	0.864 inch^2	Flow Area	0.986 inch^2
Fluid Type	Milk	Fluid Type	hot water

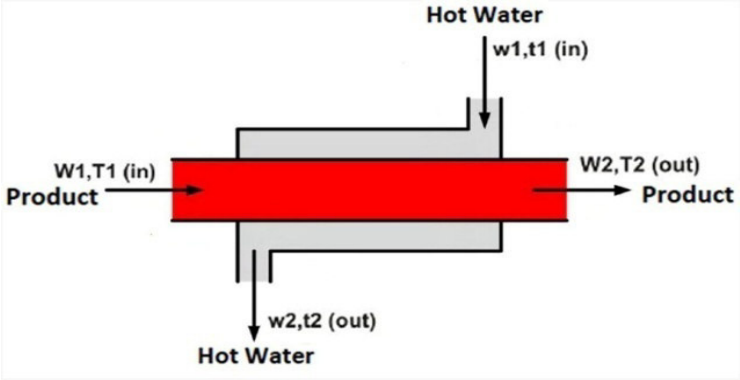


Fig. 2. *Mass flow on operating system*

2.5. The Mass Flow

The mass flow that occurs is:

$$\text{In} = \text{Out} \rightarrow \left(W_1 \cdot T_1 + w_1 \cdot t_1\right) = \left(W_2 \cdot T_2 + w_2 \cdot t_2\right) \quad (1)$$

Heat balance:

$$Q = W \cdot \int_{Tref}^{T} C_p \cdot dT \quad (2)$$

$$Q_{in} = W \cdot \int_{Tref}^{T1} \left(A + B\left(T\right) + C\left(T\right)^2 + D\left(T\right)^3\right) dT \quad (3)$$

Q absorb:

$$Q_{absorb}\left[\%\right] = \frac{Q_{tube}}{Q_{shell}} \cdot 100 \quad (4)$$

Heat loss:

$$\sum Q_{loss} = \sum Q_{in} - \sum Q_{out} \quad (5)$$

$$\sum Q_{in} = \sum Q_{in\,anulus} - \sum Q_{in\,in\,pipe} \quad (6)$$

$$\sum Q_{out} = \sum Q_{out\,anulus} - \sum Q_{out\,in\,pipe} \quad (7)$$

Log mean temperature different (LMTD):

$$LMTD = \frac{\left(T_1 - t_2\right) - \left(T_2 - t_1\right)}{\ln \frac{T_1 - t_2}{T_2 - t_1}} \quad (8)$$

Caloric temperature (T_c):

$$T_c = T_2 + F_c \cdot \left(T_1 - T_2\right) \quad (9)$$

Area of Flow (α):

1. Flow area in shell (α_s):

$$\alpha_s = \frac{\left(\frac{\pi}{4} \cdot ID_s^2\right) - \left(\frac{\pi}{4} \cdot OD_t^2 \cdot Nt\right)}{144} \quad (10)$$

2. Flow area on tube (α_t):

$$\alpha_t = \frac{N_t \cdot a't}{144 \cdot n} \quad (11)$$

Heat Transfer Coefficient ($\frac{h}{\varphi}$):

$$\frac{h_0}{\varphi_s} = J \cdot H_s \cdot \frac{k_s}{D_e} \cdot \left(Prs\right)^{1/3} \quad (12)$$

$$\frac{h_i}{\varphi_t} = J \cdot H_t \cdot \frac{k_t}{ID} \cdot \left(Prt\right)^{1/3} \quad (13)$$

Tube wall temperature (t_w):

$$t_w = t_c \cdot \frac{\frac{h_0}{\varphi_s}}{\frac{h_{io}}{\varphi_t} + \frac{h_0}{\varphi_s}} \cdot \left(T_c - t_c\right) \quad (14)$$

Overall heat transfer coefficient (U):

1. Overall heat transfer coefficient at net time

$$U_c = \frac{h_{i0} \cdot h_0}{h_{i0} + h_0} \quad (15)$$

2. Overall heat transfer coefficient during operation:

$$U_d = \frac{Q}{A \cdot \Delta T} \quad (16)$$

Resistance Dirt Factor (R_d):

$$R_d = \frac{U_c - U_d}{U_c \cdot U_d} \quad (17)$$

2.6. DNA Extraction and DNA Amplification by PCR Method

Genomic DNA extraction used gram+buffer solution, lysozyme, and proteinase enzymes. DNA binding used absolute ethanol in the GD column, washing was done using a buffer. The final step in DNA extraction was that DNA samples were amplified by the PCR method using the 16S rRNA gene. The sequences for primers were rD1 (5-AGA GTT TGA TGA TCC TGG CT C AG-3') dan fD1 (5-AAG GAG GTG ATC CAG CC-3) [3].

The amplification of each cycle consisted of a Pre-denaturation of 95°C for 3 minutes 1 cycle, a denaturation of 95°C for 15 sec, an annealing of 50°C for 15 seconds, an extension of 72°C for 15 seconds, and a final extension of 72°C for 1 minute.

PCR product detection was performed by electrophoresis using a 1.5% agarose gel. Positive results were indicated by the presence of a DNA band aligning with the 1300 bp marker, while negative results were indicated by the absence of a band on the gel.

2.7. DNA Sequencing

In PCR products from samples that showed positive electrophoresis, results were carried out through DNA sequencing, 16S rRNA coding DNA sequence analysis using BLAST nucleotide sequences from the results of 16S rRNA sequencing with a database that is used to compare the nucleotide sequences of the samples. 16S rRNA sequencing results with the database available on the site www.ncbi.nlm.nih.gov [24], which is used to find the similarity of nucleotide or protein sequences. Alignment and kinship visualization using a phylogenetic tree based on the "neighbor-joining method" was performed using the MEGA program.

2.8. Statistical Test

The ANOVA test was used to determine the average value, range of variation, and standard deviation. Meanwhile, the posthoc Tukey LSD test was used to see differences in Rd and the number of bacteria.

3. Results

3.1. Heat Balance on the Formation of Resistance Dirt Factor (Rd)

Table 3 shows the results of the heat balance calculations involved in each batch. The observed heat balance is used to obtain the Rd value formed. There was no significant difference ($p>0.05$) in the results of Δ_t (6.59-6.94°F). It did not significantly affect the difference in Rd (0.0076-0.0091 $H.Ft^2.F.Btu^{-1}$) and it also applies to Q_{in}, U_c, and U_d.

Significant difference test of heat balance (mean ± std. deviation) Table 3

Batch	H_o [$Btu.H^{-1} ft^{-2}.F^{-1}$]	H_{io} [$Btu.H^{-1} ft^{-2}.F^{-1}$]	Δ_t [°F]	Q_{in} [$Btu.h^{-1}$]	U_c [$Btu.H^{-1} ft^{-2}.F^{-1}$]	U_d [$Btu.H^{-1} ft^{-2}.F^{-1}$]	R_d [$H.Ft^2.F.Btu^{-1}$]
1904001	473.95 ± 4^{b}	394.01 ± 5^{b}	6.59 ± 0.56^{a}	179381.76 ±1200^{a}	355.63 ± 10^{a}	107.74 ± 5^{b}	0.0076 ± 0.001^{a}
1904002	468.13 ± 8^{ab}	389.17 ± 4^{ab}	6.94 ± 0.48^{a}	188960.01 ± 1500^{c}	351.68 ± 15^{a}	117.54 ± 3^{c}	0.0081 ± 0. 001^{a}
1904003	474.61 ± 5^{b}	394.56 ± 4^{b}	6.75 ± 0.52^{a}	183871.61 ± 1240^{b}	356.06 ± 13^{a}	99.89 ± 4^{a}	0.0087 ± 0.002^{a}
1904004	460.93 ± 6^{a}	383.18 ± 4^{a}	6.94± 0.36^{a}	188960.01 ± 1680^{c}	346.78 ± 16^{a}	105.37 ± 4^{ab}	0.0076 ± 0.0012^{a}
1904005	475.33 ± 5^{b}	395.16 ± 8^{b}	6.94 ± 0.56^{a}	188960.01 ± 1730^{c}	356.54 ± 12^{a}	107.46 ± 3^{b}	0.0091 ± 0.001^{a}

Note: Anovatest_Tukey LSD posthoc using $p<0.05$. The Superscript (letter) was the same, indicating no significant difference. Observations were made in 5 batches where each batch was processed for 110 minutes and the fouling was removed and then continued to process the next batch. Cleaning of each batch was done to obtain average data for each fouling forming factor. The test was repeated three times.

3.2. The Relationship between the Overall Net Heat Transfer Coefficient (U_c) and Operating (U_d)

The amount of R_d was formed due to these two variables, i.e. the condition Uc which was influenced by the heat transfer coefficient ($\frac{h}{\varphi}$) on the flow in the shell and tube. Meanwhile, the condition of U_d was highly dependent on the incoming heat (Q_{in}), the area of flow in the shell (α_s) and the tube and the temperature difference (Δ_t). The relationship between the two variables in each batch was:

The overall heat transfer coefficient at net time (U_c) in batch 190401 (Figure 3a) was stable from the beginning to minute 70 in the range of 356-358 $Btu.H^{-1}ft^{-2.}F^{-1}$ then decreased slowly to a limit of 352 $Btu.H^{-1}ft^{-2.}F^{-1}$. The reverse condition is shown by U_d in the form of a decrease from 170 to 60 $Btu.H^{-1}ft^{-2.}F^{-1}$.

The overall heat transfer coefficient at net time (U_c) in batch 190402 (Figure 3b) was stable from the beginning to minute 70 with an average of 352 $Btu.H^{-1}ft^{-2.}F^{-1}$ then it decreased slowly to a limit of 349 $Btu.H^{-1}ft^{-2.}F^{-1}$ and increased in minute 95 to 354.5 $Btu.H^{-1}ft^{-2.}F^{-1}$ and decreased to a limit of 351 $Btu.H^{-1}ft^{-2.}F^{-1}$. The overall heat transfer coefficient at operation (U_d) decreased continuously in 30 minutes from 110 to 50 $Btu.H^{-1}ft^{-2.}F^{-1}$ at the end of the process.

Batch 190403 showed almost the same phenomenon (Figure 3c) where there was a consistent decrease in U_d conditions, namely 170 to 50 $Btu.H^{-1}ft^{-2.}F^{-1}$. Stability occurs under U_c conditions in the range of 356-357 $Btu.H^{-1}ft^{-2.}F^{-1}$ and decreased to 353 $Btu.H^{-1}ft^{-2.}F^{-1}$.

In batch 190404 (Figure 3d) the decrease occurred in U_d conditions from 180 to 60 $Btu.H^{-1}ft^{-2.}F^{-1}$. The condition of U^c stability was seen until minute 70 ranging from 346-348 $Btu.H^{-1}ft^{-2.}F^{-1}$ and experienced the lowest decrease in minute 78 of 344 $Btu.H^{-1}ft^{-2.}F^{-1}$ and increased at the level of 350 $Btu.H^{-1}ft^{-2.}F^{-1}$ in minute 100.

In batch 190405 (Figure 3e) the overall heat transfer coefficient at net time (U_c) was stable in the range of 356-358 $Btu.H^{-1}ft^{-2.}F^{-1}$ at the beginning of the process until minute 70. The decrease occurred at minute 79 by 354 $Btu.H^{-1}ft^{-2.}F^{-1}$ and experienced the highest increase of 359 $Btu.H^{-1}ft^{-2.}F^{-1}$ in minute 100. U_d conditions consistently decreased from 250 to the level of 50 $Btu.H^{-1}ft^{-2.}F^{-1}$.

The relationship between mean of U_d and U_c (Figure 3f) was shown. In terms of phenomena at U_c, the lowest value occurred in batch 190404, namely 346 $Btu.H^{-1}ft^{-2.}F^{-1}$, and the highest value in batch 190405 was around 356 $Btu.H^{-1}ft^{-2.}F^{-1}$. In the case of U_d, the highest value in batch 190404 was 65 $Btu.H^{-1}ft^{-2.}F^{-1}$ and the lowest was seen in batches 1904002 and 190405 at 42 $Btu.H^{-1}ft^{-2.}F^{-1}$.

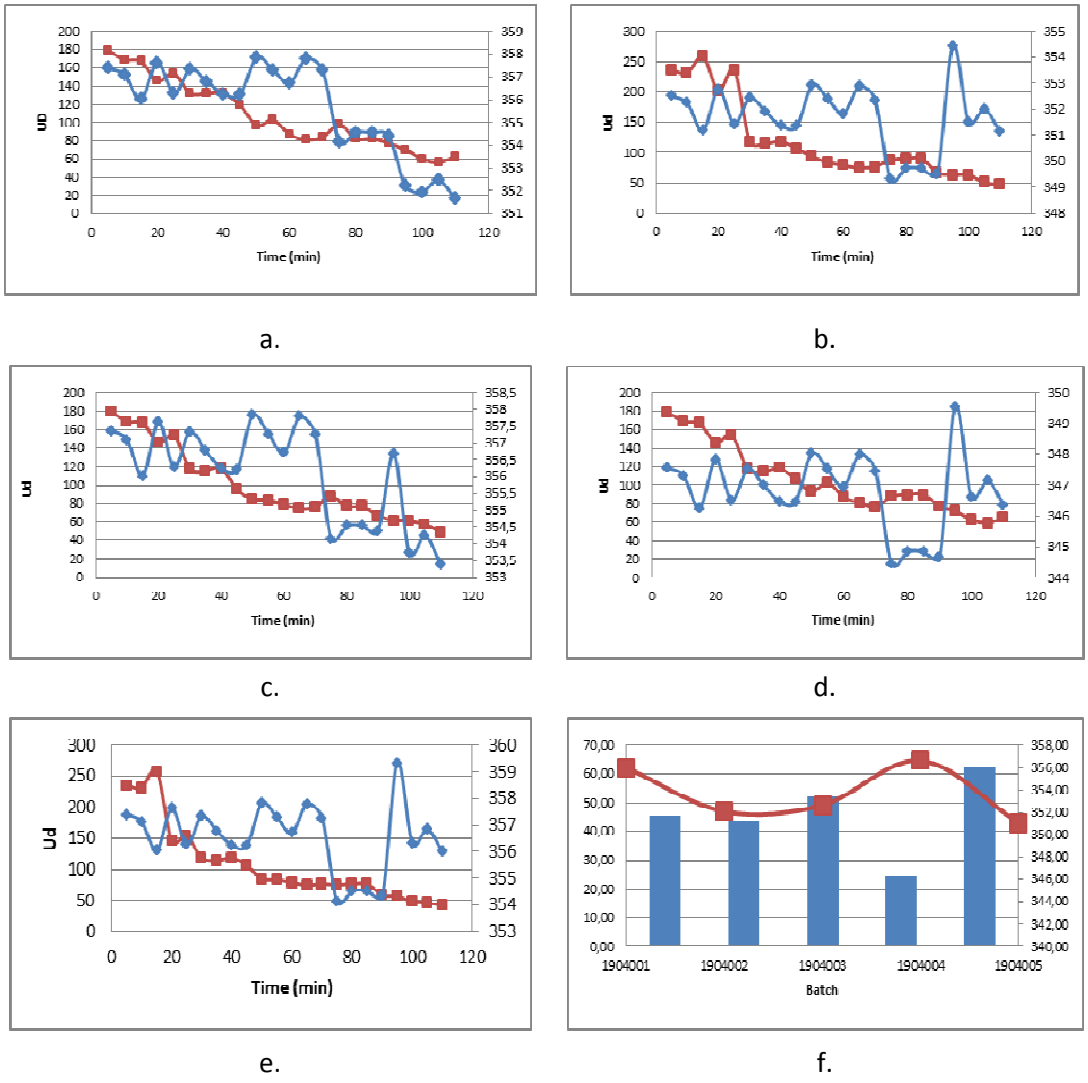


Fig. 3. *The relationship between Uc (with blue colour) and Ud (with red colour): a. batch no. 1904001; b. batch no. 1904002; c. batch no. 1904003; d. batch no. 1904004; e. batch no. 1904005; f. the average of ID and UC of the five batches*

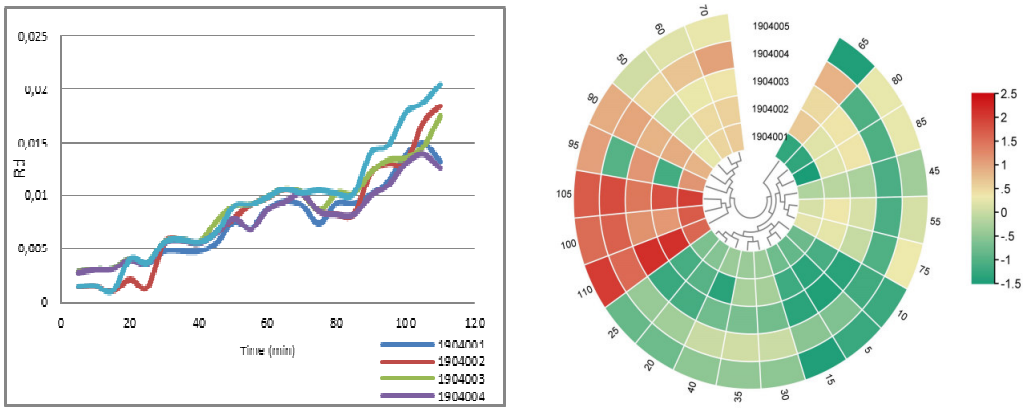
3.3. Resistance Dirt Factor (R_d)

The resistance dirt factor (R_d) is formed from the correlation between U_d and U_c, based on the data above, the value of R_d can be seen in Figure 4.

The formation of the R_d layer increased per unit time (minutes) in each batch (Figure 4a). This indicates that the protein and minerals contained in the milk have been deposited in the pipe lining. The measurement of the R_d value was carried out in the UHT area. The formation of the highest R_d in batch 190405 was 0.0205 $h.Ft^2.F.Btu^{-1}$ at minute 110. At the same time, the lowest R_d was seen in batch 190404 at 0.0126 $h.Ft^2.F.Btu^{-1}$. In this case, R_d is highly influenced by time. with no

impurity initially. R_d occurs until the equilibrium point where the fouling removal rate is linear with the deposition rate. R_d keeps increasing so it requires some cleaning effort at some point.

Determination of critical point of operation based on scale (Figure 4b). The maximum standard R_d in operation time, in the form of scale (1.5) showed that the ideal operation condition was >1.5. In this study, all four batches showed that the initial critical point (scale 1.5) was at 105 minutes. In this study, the four batches showed the beginning of the critical point (scale of 1.5) at 105 minutes. At 110 minutes the scale value of batches 01 and 04 was still within limits. In this condition it makes it easier to determine the sanitation time (CIP) which is around 110 minutes but the R_d value at 110 minutes must be analyzed for the amount of pathogenic bacterial contamination.



a. b.

Fig. 4. *The formation of Rd per unit time: a. determination of the Rd value (H.Ft².F.Btu⁻¹) during the sterilization process for 110 minutes; b. mapping of the Rd values for each batch based on the process time*

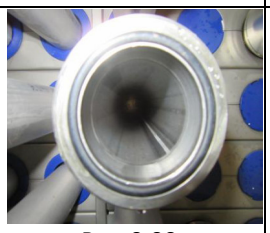
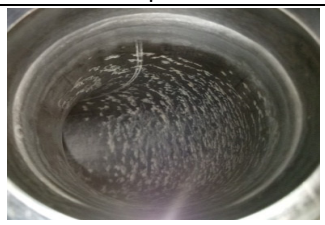
3.4. The Effect of Resistance Dirt Factor on the Growth of Microorganisms

3.4.1. The Final Product After the UHT Process

The relationship between the number of bacteria in the final product and the R_d value, during the sterilization process was studied. We grouped the R_d visualisation into 3 categories, namely fouling at R_d = 0.0126 -0.0205 in the UHT area, R_d = 0.005 - 0.006 (distribution pipe area after the UHT process), and R_d = 0.00 h. Ft^2. F. Btu^{-1} (Rd in UHT area after CIP).

The effect of the resistance dirt factor on the growth of microorganisms (Table 4), at Rd 0.0205 h.Ft^2.F.Btu^{-1}, the total plate count of bacteria is outside the standard allowed (standard < 10 cfu/mL), the standard reference refers to SNI 7388:2009 [9]. The CIP reference is based on the value of Rd ≤ 0.0205 h.Ft^2.F.Btu-^{1}, for 110 minutes, if one batch takes 60 minutes, then every two batches must be carried out in the CIP process.

The effect of resistance dirt factor on the growth of microorganisms Table 4

The number of bacteria [Cfu.ml^{-1}]	R_d = 0.005	R_d = 0.006	R_d = 0.0126	R_d = 0.0132	R_d = 0.0175	R_d = 0.0184	R_d = 0.0205
Total plate count [Cfu.mL^{-1}]	<10	<10	<10	<10	<10	<10	>20
Area	Rd in the UHT area after CIP		Rd in distribution pipe area after UHT process			Rd in UHT area	
Physical appearance	 R_d = 0.00 h.Ft^2.F.Btu^{-1}		 R_d = 0.005 – 0.006 h.Ft^2.F.Btu^{-1}			 R_d = 0.0126 – 0.0205 h.Ft^2.F.Btu^{-1}	

3.4.2. Microbiological Tests on the Distribution Pipes of the UHT Area

Microbiological tests on the distribution pipes of the UHT area when R_d is around 0.0205 were performed. In this test, the pipes are removed, then tested for the number of bacteria based on time (every hour). The complete results of the qualitative and quantitative tests of bacteria can be seen in Table 5 and Figure 5.

At the first and second hour there was no significant effect ($p>0.05$) on the growth of pathogenic bacteria, but it had a significant effect ($p<0.05$) on lactic acid bacteria. The time of 3 h and 4 h had a significant effect ($p<0.05$) on some pathogenic bacteria (*Pseudomonas* sp., *Enterobacter* sp.), the rest had no significant effect. At this time, the effect was significant ($p<0.05$) for lactic acid bacteria.

The 5th and 6th hour had no significant effect ($p>0.05$) on pathogenic bacteria (*Streptococcus* sp., *Enterobacter* sp., *Corynebacterium* sp. *Bacillus* sp.), the rest had a significant effect. This condition had a significant effect ($p<0.05$) on all lactic acid bacteria. The highest Time (7 h) had a significant effect ($p<0.05$) on all pathogenic bacteria and lactic acid bacteria.

At the DNA sequencing stage based on protein bases, 15 bacterial species were found, which we divided into two groups, namely: lactic acid bacteria (L) and pathogenic bacteria (P). Referring to Figures 5a and 5c, eight species of pathogenic bacteria and seven lactic acid bacteria were found.

The number of bacteria in the UHT pipe fouling layer area at R_d=0.0205 Table 5

The number of bacteria [Cfu.ml^{-1}]	1 h	2 h	3 h	4 h	5 h	6 h	7 h
Streptococcus sp.	0.00a	0.00^{a}	53.33 ± 5.77^{b}	68.33 ± 2.58^{b}	81.67 ± 2.88^{c}	108.33 ± 2.8^{c}	138.33 ± 2.81^{d}
Staphylococcus sp.	0.00a	0.00^{a}	203.33 ± 5.78^{b}	223.33 ± 5.79^{c}	258.33 ± 2.80^{d}	286.67 ± 5.81^{e}	330.0 ± 10.0^{f}
Pseudomonas sp.	0.00a	0.00^{a}	203.33 ± 5.79^{b}	236.33 ± 5.80^{c}	278.33 ± 2.81^{d}	318.33 ± 2.82^{e}	361.67 ± 2.88^{f}
Micrococcus sp	0.00a	0.00^{a}	101.67 ± 2.80^{b}	118.33 ± 2.88^{c}	138.33 ± 2.86^{d}	158.33 ± 2.88^{e}	178.33 ± 2.88^{f}
Enterobacter sp.	0.00a	0.00^{a}	106.67 ± 11.5^{b}	123.33 ± 5.7^{b}	143.33 ± 5.7^{c}	156.67 ± 5.7^{c}	176.67 ± 5.9^{d}
Corynebacterium sp.	0.00a	0.00^{a}	40.00 ± 0^{b}	60.00 ± 0^{c}	80.33 ± 5.7^{d}	90.67 ± 5.7^{d}	105.67 ± 2.8^{e}
Bacillus sp.	0.00a	0.00^{a}	40.00 ± 0^{b}	40.00 ± 0^{b}	40.00 ± 0^{b}	40.00 ± 0^{b}	60.00 ± 0.5^{c}
Actinomycetes sp.	0.00a	0.00^{a}	70.00 ± 0^{b}	80.00 ± 2^{b}	100.00 ± 0^{c}	130.00 ± 10^{d}	160.00 ± 0^{e}
Lactococcus lactis	1303 ± 57.7^{a}	2068 ± 54.7^{b}	2903 ± 57.7^{c}	3716.67 ± 57.7d	4503 ± 57.4^{e}	5283 ± 28.8^{f}	6066.67 ± 57.7^{g}
Lactiplantibacillus plantarum	2266 ± 50^{a}	4283 ± 28^{b}	6316 ± 57^{c}	8283± 28d	10300 ± 100^{e}	12300 ± 100^{f}	14300 ± 100^{g}
Lactobacillus	4650 ± 50^{a}	5643 ± 50^{b}	6683± 70^{c}	7710 ± 60d	8716 ± 70^{e}	9683 ± 70^{f}	10560 ± 80^{g}
Leuconostoc falkenbergense	2150 ± 50^{a}	3116 ± 50^{b}	4116 ± 60c	5066 ± 50d	6116 ± 70^{e}	7116 ± 70^{f}	8116 ± 60^{g}
Lactococcus lactis subsp. cremoris C4	1216 ±30^{a}	2233 ±40^{b}	3300 ±100c	4483 ±100d	5566 ±60^{e}	6716 ±90^{f}	7783 ±80^{g}
Lactobacillus acidophilus	1183 ±30^{a}	2140 ±60^{b}	3033 ±30c	3933 ±30d	4836 ±60^{e}	5746 ±60f	6660 ±30^{g}
Lactobacillus delbrueckii	4003 ±30^{a}	5126 ±30^{b}	6256 ±30c	7380 ±40d	8490 ±80^{e}	9583 ±90^{f}	10730 ±100^{g}

Note: Anova_Tukey HSD posthoc test using $p<0.05$. The same superscript (letter) indicates no significant difference.

The quantitative test of bacteria has been shown in Table 5, but we also show the number in Figure 5b in different units (Log Cfu.ml^{-1}), and our concentration shown in Figure 5b is the bacterial species proximity test based on the neighbor-joining method in the form of a phylogenic tree (in the center of the circle).

Types and names of bacteria based on protein base sequence matching with NCBI using BLAST system were also presented. In Figure 5c, we also display the access number for bacterial species, we selected bacterial matches based on an > 95% similarity.

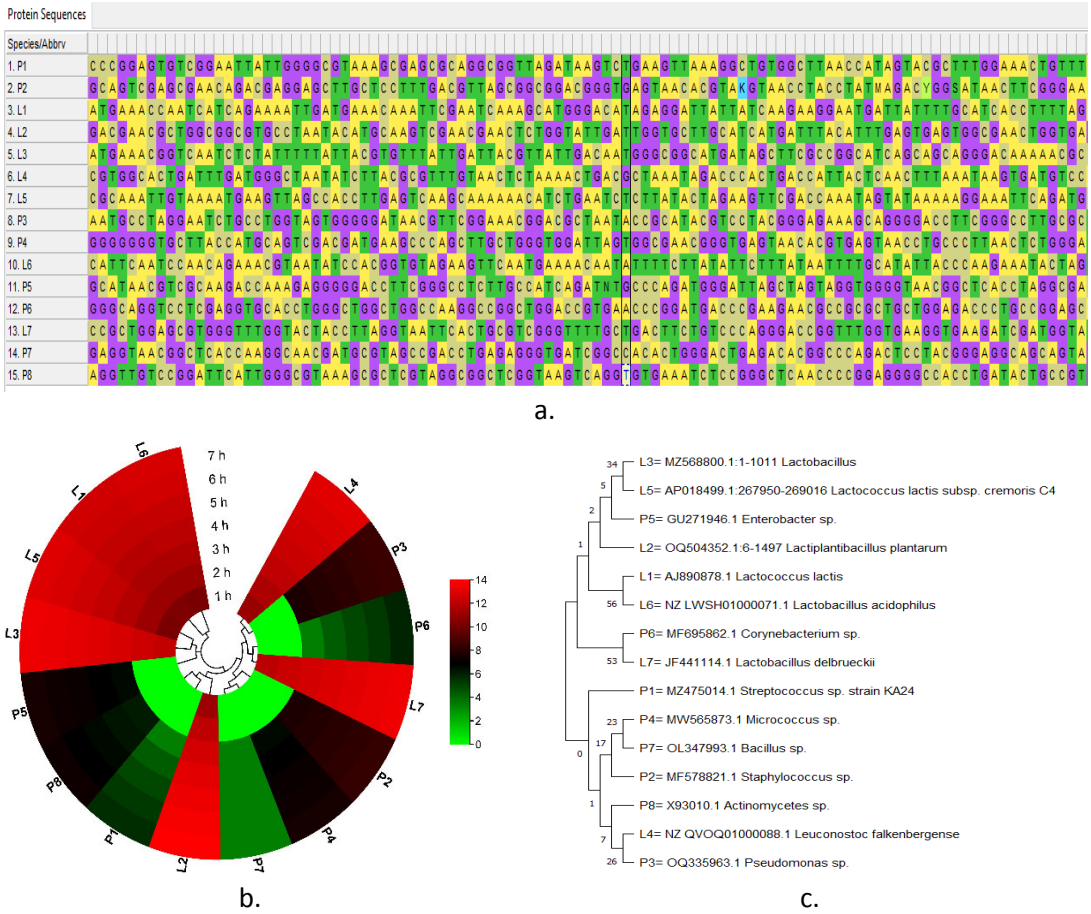


Fig. 5. Qualitative and quantitative microbiological tests: a. the protein base sequencing in the genomes of lactic acid (L) and pathogenic (P) bacteria; b. the number of bacteria in Log Cfu.ml^{-1}, in the centre of the circle is the phylogenic tree based on the neighbor-joining method; c. the names of bacteria based on matches with NCBI data by BLAST system

4. Discussion

During the UHT sterilization process, the R_d value (0.0205) was the highest in this study, indicating a total bacterial contamination of >20 Cfu.ml^{-1}. This number has exceeded the threshold allowed in Indonesia (total plate count <10 Cfu.ml^{-1}). The Rd value requires a processing time of around 110 minutes, so cleaning with the CIP method must be done every two hours of operation.

Testing the number of contaminants in the fouling layer on UHT pipes when they are not operating aims to see the effect of time on the number of bacteria in the fouling layer, whether there is engine damage or non-functioning heating in UHT. The results of the analysis show that time has a significant effect ($p<0.05$) on the number of lactic acid bacteria. Time (1 h and 2 h) had no significant effect

(p>0.05) on pathogenic bacteria, but had an effect in the range of 3 – 7 h.

This phenomenon (non-functional heating in UHT) will have an impact on the rapid growth of bacteria. So that in the event of engine damage, CIP must be carried out even though the Rd value is not maximal (0.0205).

In Figure 5, pathogenic bacteria were dominated by *Pseudomonas sp*. (200-361 CFU. ml^{-1}) and *Staphylococcus sp*. (200-330 CFU.ml^{-1}). The second domination was by *Micrococcus sp*., *Enterobacteriaceae sp*., and *Actinomycetes sp*. The third domination was *Streptococcus sp., Corynebacterium sp*. and *Bacillus sp*. The most common bacterial pathogens were: *Staphylococcus* (200-330 Cfu.ml^{-1}) which caused both subclinical and chronic mastitis [22]; *Pseudomonas* (200-361 Cfu.ml^{-1}) which causes the breakdown of proteins into amino acids and breaks down fats with lipase enzymes [19].

The same destructive properties are also possessed by *Micrococcus* and *Bacillus*. *Enterobacter* causing hydrocephalus, sepsis, necrotizing *enterocolitis* [15] was also found at < 200 Cfu.ml^{-1}. *Actinomycetes* (70-160 Cfu.ml^{-1}) decompose organic compounds such as chitin and complex sugars [2]; *Corynebacterium* sp. (<170 Cfu.ml^{-1}) is an antagonistic bacterium with a convex elevation shape and a cloudy milk chocolate colour [21].

Lactobacillus is a beneficial bacteria in dairy products [18] . A range of 1000-6000 Cfu.ml^{-1} was found for the five batches operated. Lactic acid bacteria have a preservation effect because they produce compounds that can inhibit the growth of various microbes. Most of the antimicrobial effects are due to the formation of lactic acid, acetic acid, hydrogen peroxide, diacetyl, carbon. peroxide, carbon dioxide, reuterin and bacteriocins [13].

The finding of pathogenic bacteria is an indicator for cleaning the formed fouling. CIP activity refers to Lalande et al. [12] where in general the stages of CIP equipment are as follows: a) rinsing with water for 300 seconds at 35°C to clean the remaining milk; b) alkaline rinsing (NaOH:1.5%) for 1200 seconds at 85°C is effective to clean the remaining denatured fat and protein stuck in the pipe; c) 300 seconds 35°C water rinse to clean the remaining lye; d) acid flushing (HNO_3:1%) for 900 seconds at 65°C is effective to clean the mineral scale adhering to the pipe; e) rinse water for 300 seconds at35°C to clean the remaining acid left in the pipe.

This study also analyzed the time wasted during CIP (Cleaning in Place) processes to find ways to reduce cleaning time by performing only alkaline rinsing. The decision to use only alkaline rinsing depends on the milk composition, primarily consisting of fat, protein, and sugar. Notably, minerals make up only 0.2-0.3% of the milk composition. Therefore, continuous CIP is not recommended because the minerals that adhere cannot be cleaned effectively with only alkaline solutions. The steps for alkaline CIP are as follows:

a) 300 seconds of water rinse at 35°C;
b) Alkaline rinsing (NaOH: 1.5%) for 1200 seconds at 85°C;
c) 300 seconds of water rinsing at 35°C.

This finding corroborates the results of previous studies [8, 11].

The recommended sanitization for this case is a batch change from 190401 to 190402 which is simply done with alkaline CIP. Total CIP is carried out once every 2

batches (batch 2 to 3) and so on. This strategy is an effort to get maximum sterilization by optimizing the time in operation. Physically, the thickness of fouling before and after CIP is shown in Table 4.

The CIP process gives optimal results as shown in Table 4 but the CIP process must also pay attention to the composition of the raw materials used so that the results are more optimal. The sterilization reference is based on the amount of microorganism contamination and the Rd which has reached 0.0126 – 0.0205 $h.Ft^2.F.Btu^{-1}$ continues to be developed until optimal results are obtained with continuous evaluation and innovation.

5. Conclusion

During the UHT sterilization process, the Rd value (0.0205 $h.Ft^2.F.Btu^{-1}$) had an impact on total bacterial contamination > 20 $Cfu.mL^{-1}$. This number has exceeded the threshold allowed in Indonesia (total plate count < 10 $Cfu.mL^{-1}$).

In the event of engine damage or malfunction of the heater on the UHT, time had a significant effect ($p<0.05$) on the number of lactic acid bacteria in the fouling layer in UHT pipes but the number of pathogenic bacteria was significantly different ($p<0.05$) in the range of 3 – 7 hours.

The cleaning schedule should always take into account the Resistance Dirt Factor (R_d) value and the amount of microorganism contamination that occurs. The raw material composition of processed milk is also the basis for determining Cleaning in Place (CIP). Not all batches should be treated with Total CIP, but it can be combined with alkaline CIP if the mineral value is around 0.3%.

References

1. Angeletti, S., Moresi, M., 1983. Modelling of multiple-effect falling-film evaporators. In: International Jurnal of Food Science and Technology, vol. 18(5), pp. 539-563. DOI: 10.1111/j.1365-2621.1983.tb00296.x.
2. Balachandran, C., Duraipandiyan, V., Ignacimuthu, S., 2012. Purification and characterization of protease enzyme from actinomycetes and its cytotoxic effect on cancer cell line (A549). In: Asian Pacific Journal of Tropical Biomedicine, vol. 2(2), Supplement, pp. S1138-S1146. DOI: 10.1016/S2221-1691(12)60374-8
3. Benson, D.R., Stephens, D.W., Clawson, M.L. et al., 1996. Amplification of 16S rRNA genes from Frankia strains in root nodules of *Ceanothus griseus*, *Coriaria arborea*, *Coriaria plumosa*, *Discariatoumatou*, and *Purshia tridentata*. In: Applied and Environmental Microbiology, vol. 62(8), pp. 2904-2909. DOI: 10.1128/AEM.62.8.2904-2909.1996.
4. Budianto, B., Feri, Z.O., Suparmi, A. et al., 2023. Effect of the chemical composition of fluid foods on the rate of fouling processing during sterilization. In: Vitae, vol. 30(1), pp. 1-13. DOI: 10.17533/udea.vitae.v30n1a349368.
5. Deeth, H., 2010. Improving UHT processing and UHT milk products. In: Improving the Safety and Qualitu of Milk, Elsevier Ltd, pp. 302-329. DOI: 10.1533/9781845699420.4.302.
6. Fernández García, L., Álvarez Blanco, S., Riera Rodríguez, F.A.,

2013. Microfiltration applied to dairy streams: removal of bacteria. In: Jurnal of the Science of Food and Agriculture, vol. 93(2), pp. 187-196. DOI: 10.1002/jsfa.5935.

7. Grijspeerdt, K., Mortier, L., De Block, J. et al., 2004. Applications of modelling to optimise ultra high temperature milk heat exchangers with respect to fouling. In: Food Control, vol. 15(2), pp. 117-130. DOI: 10.1016/S0956-7135(03)00023-9.
8. Hagsten, C., Innings, F., Trägårdh, C. et al., 2019. Removal of UHT dairy fouling — An efficient cleaning process by optimizing the rate controlling alkaline cleaning step. In: Food and Bioproducts Processing, vol. 113, pp. 101-107. DOI: 10.1016/j.fbp.2018.11.010.
9. Indonesia, S.N., 2009. Batas maksimum cemaran mikroba dalam pangan. SNI, 7388.
10. Indumathy, M., Sobana, S., Panda, R.C., 2021. Modelling of fouling in a plate heat exchanger with high temperature pasteurisation process. In: Applied Thermal Engineering, vol. 189, ID article 116674. DOI: 10.1016/j.applthermaleng.2021.116674.
11. Kananeh, A.B., Scharnbeck, E., Kück, U.D. et al., 2010. Reduction of milk fouling inside gasketed plate heat exchanger using nano-coatings. In: Food and Bioproducts Processing, vol. 88(4), pp. 349-356. DOI: 10.1016/j.fbp.2010.09.010.
12. Lalande, M., Rene, F., Tissier, J.P., 1989. Fouling and its control in heat exchangers in the dairy industry. In: Biofouling, vol. 1(3), pp. 233-250. DOI: 10.1080/08927018909378111.
13. Lanzanova, M., Mucchetti, G., Neviani, E., 1993. Analysis of conductance changes as a growth index of lactic acid bacteria in milk. In: Journal of Dairy Science, vol. 76(1), pp. 20-28. DOI: 10.3168/jds.S0022-0302(93)77319-1.
14. Moyes, R.B., Reynolds, J., Breakwell, D.P., 2009. Preliminary staining of bacteria: negative stain. In: Current Protocols in Microbiology, vol. 15(1), Appendix 3F. DOI: 10.1002/9780471729259.mca03fs15.
15. Odenthal, S., Akineden, Ö, Usleber, E., 2016. Extended-spectrum β-lactamase producing *Enterobacteriaceae* in bulk tank milk from German dairy farms. In: International Journal of Food Microbiology, vol. 238, pp. 72-78. DOI: 10.1016/j.ijfoodmicro.2016.08.036.
16. Sadeghinezhad, E., Kazi, S.N., Badarudin, A. et al., 2013. A review of milk fouling on heat exchanger surfaces. In: Reviews in Chemical Engineering, vol. 29(3), pp. 169-188. DOI: 10.1515/revce-2013-0003.
17. Sahoo, P.K., Ansari, I.A., Datta, A.K., 2005. Milk fouling simulation in helical triple tube heat exchanger. In: Journal of Food Engineering, vol. 69(2), pp. 235-244. DOI: 10.1016/j.jfoodeng.2004.08.014.
18. So, S., Wanapat, M., Cherdthong, A., 2021. Effect of sugarcane bagasse as industrial by-products treated with *Lactobacillus casei* TH14, cellulase and molasses on feed utilization, ruminal ecology and milk production of mid-lactating Holstein Friesian cows. In: Journal of the

Science of Food and Agriculture, vol. 101(11), pp. 4481-4489. DOI: 10.1002/jsfa.11087.

19. Stoeckel, M., Lidolt, M., Achberger, V. et al., 2016. Growth of *Pseudomonas weihenstephanensis*, *Pseudomonas proteolytica* and *Pseudomonas sp.* in raw milk: Impact of residual heat-stable enzyme activity on stability of UHT milk during shelf-life. In: Internationl Dairy Jurnal, vol. 59, pp. 20-28. DOI: 10.1016/j.idairyj.2016.02.045.
20. Suhengki, H., Lubis, A.F., 2018. Pengaruh fouling terhadap laju perpindahan panas. In: Jurnal of Power Plant, vol. 6(1), pp. 48-57.
21. Taponen, S., Liski, E., Heikkilä, A.-M. et al., 2017. Factors associated with intramammary infection in dairy cows caused by coagulase-negative staphylococci, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Corynebacterium bovis*, or *Escherichia coli*. In: Journal of Dairy Sciene, vol. 100(1), pp. 493-503. DOI: 10.3168/jds.2016-11465.
22. Valckenier, D., Piepers, S., Schukken, Y.H. et al., 2020. Longitudinal study on the effects of intramammary infection with non-aureus staphylococci on udder health and milk production in dairy heifers. In: Jurnal of Dairy Science, vol. 104(1), pp. 899-914. DOI:10.3168/jds.2020-18685.
23. Wallhäußer, E., Hussein, M.A., Becker, T., 2012. Detection methods of fouling in heat exchangers in the food industry. In: Food Control, vol. 27(1), pp. 1-10. DOI: 10.1016/j.foodcont.2012.02.033.
24. www.ncbi.nlm.nih.gov. Accessed on: December, 2022.
25. Yoo, J.-A., Hardin, M.T., Chen, X.D., 2006. The influence of milk composition on the growth of *Bacillus stearothermophilus*. In: Journal of Food Engineering, vol. 77(1), pp. 96-102. DOI: 10.1016/j.jfoodeng.2005.06.053.

Nomenclature

Symbol	Name [measure units]	Symbol	Name [measure units]
c	Index for cold fluid	σ	proportional constant [BTU/hour ft^2 °C]
s	Index for shell part	m	flow rate of hot fluid flow [lb/hour]
t	Index for tube section	C_p	specific heat coefficient [BTU/lb °F]
Q	Heat transfer rate [BTU/hour]	F_c	caloric fraction
K	Thermal conductivity [BTU/hour]	ID	inside diameter [ft]
A	Cross-sectional area of heat transfer [ft^2]	OD	outside diameter [in]
T	Temperature [°F]	C	distance between tubes [in]
x	Heat flow path distance [ft]	B	distance between baffles [in]
h	Heat transfer coefficient [BTU/hour ft^2 °C]	P	pitch [in]
e	Emissivity (0 to 1)	a	flow area [ft^2]
Nt	number of tubes	N	number of passes
G	fluid flow pressure [lb/ft^2]	De	equivalent diameter [ft]
μ	viscosity [lb/h ft]	ϕ	Ratio of viscosity
h_{i0}	the shell heat transfer coefficient [Btu/(h) (ft^2)(F)]	h_0	the tube heat transfer coefficient [Btu/(h) (ft^2)(F)]
P_{rs}	prandl number in shell	P_{rt}	prandl number in tube
JH_s	Heat transfer factor in shell	JH_t	Heat transfer factor in tube
W	Mass flowrate of fluid [kmol/h]	LMTD	Log mean temperature different [°F]
G	Mass Flow Rate	*Uc*	Overall heat transfer coefficient when clean [Btu/ h.ft^2.F]
Ud	Overall heat transfer coefficient after operation [Btu/ h.ft2.F]	$(\frac{h}{\varphi})$	Heat Transfer Coefficient
t_w	Temperature on the tube wall [°F]	R_d	Fouling factor [H. Ft2. F / Btu]