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# Analysis and Optimization of the Effect of Process Parameters of Silver Anode Technique on *Pseudomonas aeruginosa* in Raw Milk

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**HIGHLIGHTS**

- Strong antibacterial and antibiofilm effects of the Silver Anode Technique were observed against *P. aeruginosa*.
- Determining the optimum parameters is important in preventing time and quality losses in terms of the dairy sector.
- Optimum process parameters for the lowest bacterial colony count were determined as 20 µA current and 60 seconds time with the Taguchi method.

**Abstract:** The present research aimed to experimentally and statistically investigate the effects of current and time parameters on the inhibition of *Pseudomonas aeruginosa* ATCC 27853 using the silver anode technique. Accordingly, cold raw milk was divided into groups and the experimental design was carried out according to the Taguchi $L_{16}$ index. Four levels of current intensity (5, 10, 15, and 20 µA) and duration (15, 30, 45, and 60 sec) were selected as the process parameters, and the bacterial colony count was chosen as the output parameter. The bacteria were inoculated into raw milk, stored in the cold and the silver anode technique was applied. After incubation *Pseudomonas aeruginosa* bacterial colony counts were determined. Experimentally-obtained bacterial colony counts were statistically analyzed using the Taguchi method, ANOVA, and regression analysis. The optimum process parameters for the lowest bacterial colony count (Nc) were 20 µA current and 60-second duration. Also according to the variance analysis results, the current intensity was the most effective process parameter on the bacterial colony counts. The regression model developed to estimate the output parameter (bacterial colony counts) yielded successful results with a high determination coefficient ($R^2$) of 96.26%.

**Keywords:** *Pseudomonas aeruginosa*; raw milk; silver anode; antibacterial effect; Taguchi.

## INTRODUCTION

Raw milk is a good source of nutrients for humans and microorganisms in terms of fats, proteins, vitamins and minerals. Therefore, raw milk can rapidly deteriorate microbiologically and chemically. It has been accepted that raw milk is sterile inside the udder. However, due to insufficient hygiene and sanitation conditions around milking environments, improper and careless milking processes, the bacterial load of raw milk [1, 2].

The primary deterioration of milk is induced by microorganisms. Most of the microorganisms in raw milk harm not only human health and beneficial microorganisms but also cause chemical and structural deterioration in the product with the metabolic residues they produce. Cold food storage is the most commonly used preservation method against microorganisms that can quickly reproduce and develop in almost any environment. It has been accepted that *Pseudomonas* group bacteria contaminate milk from farm and milking environments and are found in the natural flora of raw milk. *Pseudomonas* is among the most critical microorganisms that can cause milk spoilage by showing optimum growth under cold storage conditions [3]. *Pseudomonas aeruginosa* can rapidly generate under colder storage conditions, produce metabolic residues and synthesize temperature resistant enzymes (protease and lipase). The proteases cause protein denaturation called sweet coagulation in processed packaged milk, and lipases produced cause hydrolytic rancidity in the product by hydrolyzing the fats in the milk. It is common for *Pseudomonas aeruginosa* to be ingested by humans through contaminated foods. It is common for *Pseudomonas aeruginosa* to be ingested by humans through contaminated foods. This bacterium causes serious respiratory tract infections, cystic fibrosis disease and epidemics, especially in immunocompromised individuals and children aged 0-3 years [4, 5].

*Pseudomonas aeruginosa* is able to form biofilms having some nutritional and vital risks. Through biofilm formation and strong virulence factors, disinfectant protects itself from chemical, technical and mechanical destruction. In order to deal with *Pseudomonas aeruginosa*, numerous studies have been carried out with technical methods, including various disinfectants [6], toxic peptides [7], enzymes [8], UV rays [9] and ultrasound [10]. However, no satisfactory results have been obtained. Therefore, different alternative methods are required to struggle *Pseudomonas aeruginosa*.

Silver has been used as an antibacterial agent in food and medicine industry since 3100 BC. It has also been reported that the Sumerians made water mains pipes from silver, silver coins were thrown into milk containers in the Old West, and silver was even used to treat burns and wounds [11, 12]. In 1869, Raulin determined that *Aspergillus niger* could not grow in silver Petri dishes due to the antibacterial effect of silver and the first scientific study on this subject was carried out [12]. The action mechanism of silver on the bacterial cell comprises the bonding of silver to thiol (-SH) groups containing sulfur, oxygen and nitrogen. Thus, silver causes denaturing of bacterial proteins and even damages bacterial DNA and RNA [12]. Also, it reduces the membrane permeability of the bacterial cell, inactivates respiratory enzymes and prevents bacterial respiration, deprives the bacteria of energy and causes cell death [13, 14, 15, 16].

The ionization of silver by giving a weak and direct current is defined as the "Silver Anode Technique". It is known that even if silver does not ionize, it has an antibacterial effect around itself. However, it has been stated that the silver ions formed by the silver anode technique can reach longer distances with the driving force of the electric current, which expands the antibacterial effect area [17]. Silver anode technique is applied with a simple mechanism consisting of an anode and cathode pole, power supply and potentiometer that helps adjust the current's intensity, provided that the anode is pure silver. Electric current is supplied to the system for a certain period and ionization of the silver metal is ensured from the anode pole [18].

Suttasattakrit and coauthors [19] determined that the applications of electric currents creates a synergistic effect on the antibacterial effect of silver. The researchers obtained an effective antibacterial and antibiofilm result compared to the model without ionic silver and current. Mahmoud and El-Liethy [20] obtained a practical antibacterial effect against *E. coli* by applying current at various levels to the silver-coated electrodes in wastewater treatment. Cibik and Duran [18] provided the ionization of silver by the current on silver plates and obtained antibacterial results against *Pseudomonas spp*., *E. coli*, and lactic acid bacteria in raw milk.

Different methods and inhibitory substances are still being investigated in the fight against *Pseudomonas aeruginosa*, which is resistant to most disinfectants, antibiotics and technical processes. Silver anode technique has been lately examined in the literature as an effective technique for inhibiting *Pseudomonas aeruginosa*. Due to increasing antibiotic resistance, numerous antibacterial studies have been carried out with the silver anode technique on environmental issues such as wastewater treatment [20] in addition to those, especially in medicine [21, 22, 23] and dentistry [11, 24, 25]. As a result of these studies, effective and successful results were obtained on the inhibition of *Pseudomonas aeruginosa*.

Some field studies adopted the silver anode technique and compared the antibacterial/antibiofilm effect results against *Pseudomonas aeruginosa* for biocidal/biostatic purposes in current/non-current environments. Studies on using the silver anode technique to inhibit *Pseudomonas aeruginosa* bacteria have not yet been carried out in the food field. Again, the current/time relationship, one of this technique's parameters, has not been experimentally and numerically investigated. The present study aimed to determine the most appropriate current and duration for the silver anode technique applied to inhibit *Pseudomonas aeruginosa* bacteria in raw milk. The study also aimed to reveal which factor was more effective in inhibiting *Pseudomonas aeruginosa*. Another aim of the study was to compare the inhibition rates of *Pseudomonas aeruginosa* in silver anode-treated raw milk with both experimental and finite element analysis and to make mathematical modeling.

## MATERIAL AND METHODS

### Preparation the silver rods and with Pseudomonas aeruginosa inoculated raw milks

The raw milk used in the study was obtained from Aksaray Cattle Breeders Association in a volume of 1000 ml, under sterile conditions and delivered to the laboratory without breaking the cold chain. *Pseudomonas aeruginosa* ATCC 27853 strain to be used in microbiological analyzes was obtained from Aksaray University Food Engineering Department Microbiology Laboratory. The *Pseudomonas aeruginosa* ATCC 27853 strain, at 0.5 McFarland turbidity, was first added 0.1 ml ($1.5 \times 10^6$) to 1000 ml raw milk. The raw milk was distributed into 40-ml-sterile Petri dishes using a sterile graduated cylinder. In this way, the petri dishes were made ready for the attachment of the silver anode assembly.

In the study, 99.9% pure silver obtained from Nadir Silver LLC in the form of 5- and 10-g plates and 15-g silver in the form of beads were used. Bead-shaped silvers were welded to plate-shaped silvers in the form of stems without losing their purity. A 10-g-plate (15 mm wide, 25 mm long, 3 mm high) was used at the anode pole and a 5-g-plate (14 mm wide, 23 mm long, 1.5 mm high) was used at the cathode pole. The handle height of the plates was 8 mm and their length was 3 mm. A 1.5-volt-clock battery was used as the power source in the silver anode assembly. The red part of the clamped conductor cable was fixed on the positive side of the battery, representing the anode pole, and the black wire part representing the cathode pole, was fixed on the negative side of the battery (Figure 1).

**Figure 1.** The silver anode assembly (left), applying current to raw milk at determined time and intensity values (right).

### Application of Silver Anode Technique

According to Ohm's law, the electric current (ampere) passing through a conductive wire is directly proportional to the pressure (volts) that this current applies to the circuit and triggers the electron current. This equation (Equation 1) was used to calculate the microampere current needed in the study [26].

$$I = V \div R \quad (1)$$

In Equation 1; I: Current, V: Voltage, R: Resistance.

The required current is provided with a 5 MΩ potentiometer. For the application, the current load of the clock battery was measured as 1.412 Volts with a multimeter (UNI-T/UT50C, China). The potentiometer, according to the formulation, was set to 0.282 MΩ for 5 µA, 0.141 MΩ for 10 µA, 0.094 MΩ for 15 µA, and 0.071 MΩ for 20 µA.

The currents and durations specified in Table 1 were applied to the prepared raw milk. Immediately after the silver anode technique (Figure 1), the silver anode-treated milk was diluted and microbiologically cultivated into the media.

**Table 1.** Current and durations applied to milk with the silver anode

| **Application Currents (µA)** | **5** | **10** | **15** | **20** |
|---|---|---|---|---|
| **Application Duration (s)** | 15 | 15 | 15 | 15 |
| | 30 | 30 | 30 | 30 |
| | 45 | 45 | 45 | 45 |
| | 60 | 60 | 60 | 60 |

## Microbiological Analyses

*Pseudomonas aeruginosa* ATCC 27853 was activated in 10 ml of Tryptic Soy Broth (TSB) medium (105459, SIGMA, Canada) for 24 hours at 37 °C. The identification was carried out using the Oxidase test (+) and growth tests at 42 °C. Petri dishes used in the study were sterilized by dry sterilization at 170 °C for 1 hour. Silver plates were autoclaved at 121°C for 1 atm-pressure for 15 minutes. Cetrimide Agar (CA), a specific medium for *Pseudomonas aeruginosa*, containing 10 ml of $L^{-1}$ glycerol (TEKKİM, Turkey, 99.5%) was used (105284, MERCK, Germany). The medium was sterilized in an autoclave and poured into Petri dishes at 12.5 ml. A 0.9% sodium chloride (NaCl) solution was used for dilutions and seeding was carried out up to $10^{-4}$ on CA adopting the spreading technique. Colonies were counted after 48 hours of incubation at 37 °C, $\log_{10}$ conversion was made and the calculations were made as log cfu $mL^{-1}$. The study was carried out in triplicate.

## Chemical Analyses

The fat and moisture contents of the raw milk brought to the laboratory were calculated according to the AOAC method [27] and protein analysis according to the Kjeldahl method [27] using a correction factor of 6.38. Carbohydrate (lactose) content was performed according to the Lane-Eynon method [28], and pH analysis was performed using a pH meter (pH211, Hanna Instruments) as indicated by Coşkun and Çağlar [29]. An $Ag^{+}$ content analysis was conducted in the group to which the maximum current and duration were applied. The analysis were performed at Aksaray University Scientific and Technological Research Center adopting the method inductively coupled Plasma Mass Spectrophotometry (ICP-MS, ThermoScientific-X Series 2) [30].

## Statistical Analyses

Based on the data obtained through experimental studies, it is necessary to determine the optimum current and time parameters against specific bacteria. Thus, it will be possible to minimize the loss of time and therefore, the quality losses that may occur in milk in the integration of the silver anode technique for dairy facilities. In a similar manner, determining the optimum parameters will prevent time and material losses in antimicrobial studies.

In the present study, the factors affecting the number of bacterial colonies in milk are the intensity and duration of the applied current. These are the independent variables. Depending on these factors, the bacterial colony counts in milk may vary, and the direction of this change cannot be determined beforehand. Therefore, the bacterial colony counts in raw milk are an uncontrollable factor. The Taguchi statistical method, was created against factors that could not be controlled and caused variability in experimental studies. Accordingly, it ensures to minimize of trial-and-error losses [31, 32, 33]. The Taguchi method provides this reduction using orthogonal (vertical) experimental design tables containing all the parameters in the experiment [34]. In the present study, silver anode experiments were designed using the $L_{16}$ standard orthogonal array of the Taguchi method. To reach the optimal current values, the intensity and duration of the current were taken as the input parameters, and the colony counts were taken as the output parameter.

Also, the relationship between dependent and independent variables was defined and modeled by S/N ratio analysis, analysis of variance (ANOVA), and regression analysis. The formula in Equation 2 was used to calculate the S/N ratios and the result obtained was based on the “smallest best" approach. MiniTab 19.1 (USA) software was applied for statistical analyses.

$$n = S \div N = -10 \log\left(\frac{1}{n}\sum_{i=1}^{n} y_i^2\right) \quad (2)$$

In Equation 2; n: the number of experimental observations, $y_i$: test data.

## RESULTS AND DISCUSSİON

The chemical analysis results of the raw milk used in the study are given in Table 2.

**Table 2.** Chemical analysis results of raw milk

| | |
|---|---|
| Fat % | 3.93 ± 0.02 |
| Protein % | 3.62 ± 0.03 |
| Lactose % | 4.49 ± 0.03 |
| Moisture % | 87.27 ± 0.05 |
| pH | 6.68 ± 0.01 |

In the study, microbiological results with *Pseudomonas aeruginosa* ATCC 27853 were counted as cfu $mL^{-1}$, and $log_{10}$ conversion was performed. The average of 3 parallels was taken to make statistical calculations and the results are shown in Table 3.

**Table 3.** Colony counts of *Pseudomonas aeruginosa* (log cfu $mL^{-1}$).

| | | **Duration (s)** | | | |
|---|---|---|---|---|---|
| | | **15** | **30** | **45** | **60** |
| **Current (µA)** | **0** | 2.18 ±0.05 | 2.18 ±0.05 | 2.18 ±0.05 | 2.18 ±0.05 |
| | **5** | 1.72 ±0.03 | 1.69 ±0.01 | 1.69 ±0.01 | 1.66 ±0.04 |
| | **10** | 1.58 ±0.01 | 1.57 ±0.02 | 1.56 ±0.02 | 1.51 ±0.01 |
| | **15** | 1.42 ±0.02 | 1.41 ±0.01 | 1.40 ±0.01 | 1.39 ±0.03 |
| | **20** | 1.39 ±0.01 | 1.37 ±0.02 | 1.34 ±0.01 | 1.31 ±0.02 |

As seen in Table 3, the final bacteria count of the milk with an initial bacteria count of 2.18 log cfu $mL^{-1}$, with silver anode application of 5, 10, 15, and 20 µA for varying durations (15, 30, 45, and 60 sec) decreased to 1.31 log cfu to $mL^{-1}$.

### Analysis of S/N Ratios

A statistical calculation known as the S/N ratio was utilized to analyze and interpret the data obtained from the study. In this calculation method, S indicates the actual value given by the system and N denotes the factors affecting the test result. All values expressed as N are all variables that can lead to deviations from the desired target result [35]. The standard $L_{16}$ orthogonal table of the bacterial colony counts (Nc), which is the output parameter and the S/N ratios of the silver anode technique parameters are given in Table 4. In contrast, the S/N responses are shown in Table 5. In these tables, the parameters with the highest S/N value are considered to determine the optimum current and time parameters. The levels of the process parameters for bacterial colony counts ($N_C$) are also shown in Figure 2 in visual form. Accordingly, the point with the highest S/N ratio for each process parameter shows the optimum level. The optimum process parameters for the lowest bacterial colony count ($N_C$) were 20 µA current and 60 s duration. In the experiments performed with these parameters, the lowest bacterial colony count ($N_C$) was determined to be 1.31 log cfu $mL^{-1}$. Also, it is seen that the number of bacterial colonies decreases with the increase in applied current value and time (Figure 2).

**Table 4.** Experimental results and S/N values

| Test Id | Current ($\mu A$) | Duration ($s$) | Nc (log cfu $mL^{-1}$) | S/N ratio |
|---|---|---|---|---|
| **1** | 5 | 15 | 1.72 | -4.71057 |
| **2** | 5 | 30 | 1.69 | -4.55773 |
| **3** | 5 | 45 | 1.69 | -4.55773 |
| **4** | 5 | 60 | 1.66 | -4.40216 |
| **5** | 10 | 15 | 1.58 | -3.97314 |
| **6** | 10 | 30 | 1.57 | -3.91799 |
| **7** | 10 | 45 | 1.56 | -3.86249 |
| **8** | 10 | 60 | 1.51 | -3.57954 |
| **9** | 15 | 15 | 1.42 | -3.04577 |
| **10** | 15 | 30 | 1.41 | -2.98438 |
| **11** | 15 | 45 | 1.40 | -2.92256 |
| **12** | 15 | 60 | 1.39 | -2.86030 |
| **13** | 20 | 15 | 1.39 | -2.86030 |
| **14** | 20 | 30 | 1.37 | -2.73441 |
| **15** | 20 | 45 | 1.34 | -2.54210 |
| **16** | 20 | 60 | 1.31 | -2.34543 |

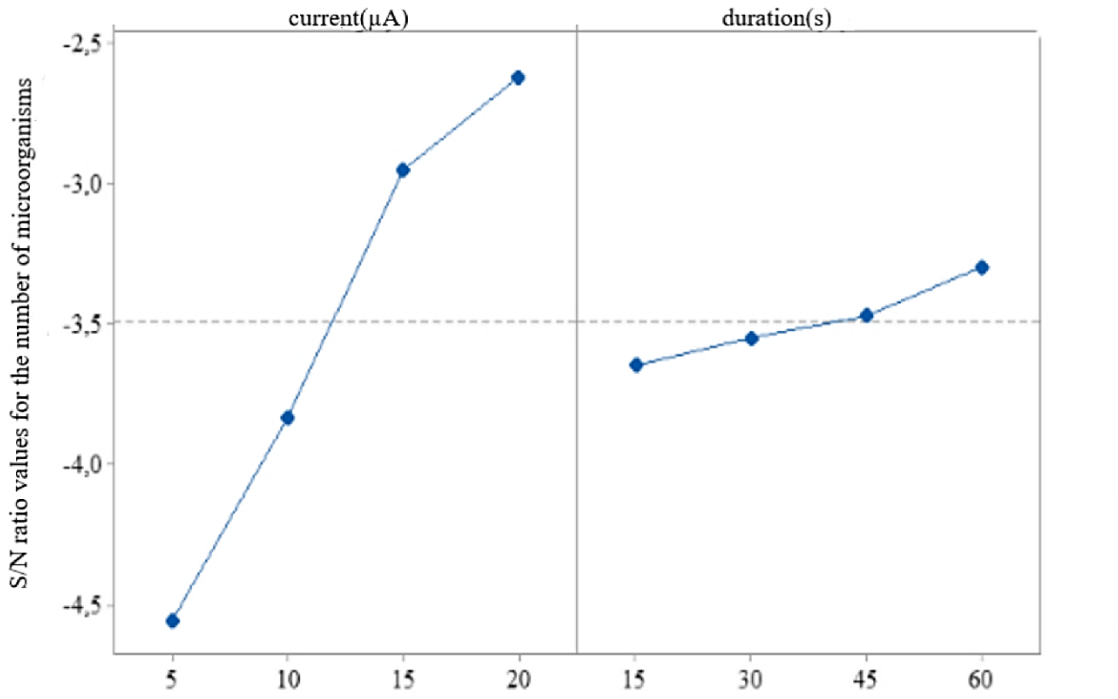


**Figure 2.** Mean distribution of S/N ratios for Nc (log cfu $mL^{-1}$)

**Table 5.** S/N response table for output parameters

| Level | Current (µA) | Duration (s) |
|---|---|---|
| **1** | -4.557 | -3.647 |
| **2** | -3.833 | -3.549 |
| **3** | -2.953 | -3.471 |
| **4** | **-2.621** | **-3.297** |
| **Delta** | **1.936** | **0.351** |

## Analysis of variance (ANOVA)

Variance analysis was performed to determine the effect levels of the silver anode technique's current intensity and time parameters on the bacterial colony count. The results are given in Table 6. In the table, the (P) value indicates significance and the (F) value indicates the effect level [36, 37]. According to the table, the parameter with the highest F value is accepted as the factor with the greatest effect on the results.

**Table 6.** Analysis of variance results

| Factors | SD | Seq SS | Adj MS | F value | P value | Impact ratio (%) |
|---|---|---|---|---|---|---|
| **Current (µA)** | 3 | 0.279619 | 0.093206 | 695.42 | 0.000 | 96.92 |
| **Duration (s)** | 3 | 0.007669 | 0.002556 | 19.07 | 0.000 | 2.66 |
| **Error** | 9 | 0.001206 | 0.000134 | | | 0.42 |
| **Total** | 15 | 0.288494 | | | | 100 |

According to the analysis of variance, current intensity and current application duration have 96.92% and 2.66% effect rates, respectively, on the bacterial colony counts (Nc). An error rate of 0.42% was determined in the Anova analysis. As a result, the most effective process parameter on the bacterial counts was the current intensity, with a contribution rate of 96.92%.

## Evaluation of the Experiment Results

Figure 3 shows the changes in the colony-forming unit counts according to the intensity and duration of the applied current in the Silver Anode Technique study conducted to reduce the *Pseudomonas aeruginosa* ATCC 27853 colony counts in raw milk.

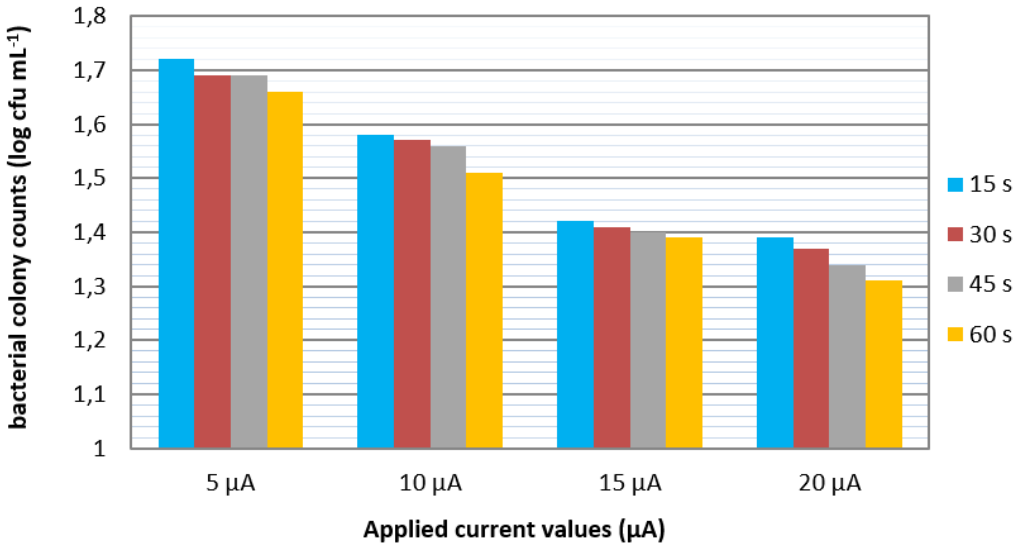


**Figure 3.** Changes in the microorganism colony count according to four currents and durations.

As seen in Figure 3, inhibition of the *Pseudomonas aeruginosa* ATCC 27853 strain in raw milk increased with the increase in current. The bacterial counts did not show a significant decrease in the varying durations of 15 seconds of the current application of 5 µA and a weak inhibition effect was achieved. Similarly, in the present parameter of 10 µA, the current was increased by five more units and applied as 10 µA, provided that the durations remained the same and a low inhibition effect was observed on the bacteria in 15-second changes. In the current applications of 15 and 20 µA, bacterial counts decreased at a low rate over time. These results confirmed that increasing current intensity is more effective than duration on bacterial inhibition. As seen in Figure 3, the lowest bacterial counts overtime at all current levels were observed at current intensities applied for 60 seconds.

The highest current intensity was determined as 20 µA and applied. In previous similar studies, no increases were observed in the antimicrobial effect with the polarization of silver electrodes at higher than 20

µA [11, 17, 18]. Also, since the application material is food, since the aim was that the silver release would not exceed the level allowed by the laws, higher current values have not been reached. The sole antimicrobial effect of silver is known. In the present study, a $Ag^+$ content analysis was carried out in the group (20 µA and 60 sec) in which the highest silver ion release was predicted and the result is given in Table 7.

**Table 7.** Ag+ contents in milk as a result of applying 20 µA current intensity for 60 seconds in the silver anode technique

| Current (µA) | Duration (sn) | $Ag^+$ content (ppb*) |
|---|---|---|
| 20 | 60 | 3.882 ± 0.015 |

ppb*: µg $L^{-1}$

The United States Environmental Protection Agency (EPA) [38] states that oral silver exposure is an average reference value of 5 µg/kg/day per person and a critical toxic exposure dose of 14 µg/kg/day per person [39]. Even the maximum amount of $Ag^+$ released was determined below the limits allowed by EPA.

The sole antimicrobial effect of silver is known. However, the silver concentrations required to kill bacteria that protect themselves in this structure by forming a biofilm, such as *Pseudomonas aeruginosa*, are 10-100-fold higher than silver sufficient to kill bacterial cells [40]. The most important physical effect of the applied current is to separate the silver ions from the silver metal and to reach the extreme points of the applied material. This is expressed as a better antibacterial result with more negative effects of silver on the bacterial cell.

Aydın [17] applied silver anode against *Pseudomonas aeruginosa* and examined the bacterial parts taken from the silver-anode-applied area with a wide inhibition zone under an electron microscope. The researcher observed that the cell structure of *Pseudomonas aeruginosa*, where silver anode was applied, formed cavities, pili-organ deformations and division defects. Wellman and coauthors [41] used the silver anode technique to prevent infections caused by biofilms in medical devices. It has been reported that the death of *Pseudomonas aeruginosa* parallels the increased current and was associated with the removal of bacterial biofilms caused by *Pseudomonas aeruginosa*. In the study, it was ineffective in killing bacteria when a current of 1 mA and antibiotics were applied together against *Pseudomonas aeruginosa*. Still, it was observed that there was an increase of approximately 7 log in bacterial death when the current was increased up to 5 mA.

Figure 4 shows the colony inoculation results of the present study. While the initial *Pseudomonas aeruginosa* ATCC 27853 strain colony load was 2.18 log cfu $mL^{-1}$ in raw milk, the colony count decreased to 1.31 log cfu $mL^{-1}$ as a result of the application of 20 µA current for 60 seconds with the silver anode technique.

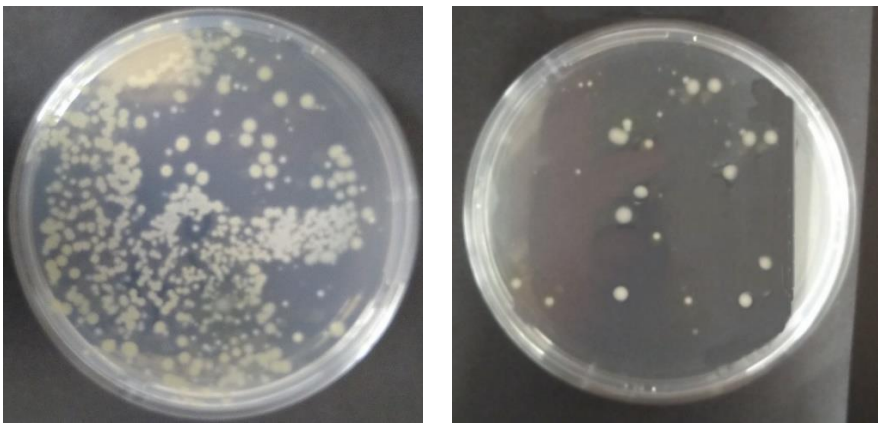

**Figure 4.** Initial *Pseudomonas aeruginosa* colony images in raw milk (left) and silver anode with 20 µA current-60 s *Pseudomonas aeruginosa* colony images after applying the technique (right)

## Regression Analysis

The mathematical expression of the relationship between the current intensity and duration, which are the inputs of the study, and the bacterial colony counts, which are the output of the study, was conducted by adopting regression analysis.

Regression analysis is a statistical calculation method used to predict the relationship between dependent and independent variables. The $R^2$ value in this method is an important indicator of the estimation performance in the relationship between the variables. $R^2$ value, which is between 0 and 100% (1) or close

to 100%, indicates that the relationship between a dependent (bacterial colony count) and independent (current intensity-current duration) variables is strongly compatible [42, 43]. In Equation 3, there is a linear equation related to the bacterial colony counts created with experimental data.

$$Nc(log\ cfu/mL) = 1.8394 - 0.02325(\mu A) - 0.001283(s) \quad (3)$$

Nc = microorganism colony counts
μA= unit of applied current
sec = current application duration

According to the linear regression equation formed with the present study's data, the $R^2$ value was determined to be 96.26%. As a result, estimation equations with a very high coefficient of determination ($R^2$) produce values close to the actual values.

**Validation Tests**

In the last stage of the study, validation experiments were carried out with optimum and random levels to check the accuracy of the optimization. To determine the success of the optimization, the confidence interval (CI) for the output parameter (Nc) was calculated using Equations 4 and 5.

$$\boldsymbol{n_{eff} = \frac{N}{1+T_{dof}}} \quad \textbf{(4)}$$

$$\boldsymbol{CI_{Nc} = \sqrt{F_{a,1,fe}\, V_e \left[\frac{1}{n_{eff}} + \frac{1}{R}\right]}} \quad (5)$$

N in Equation 4 represents the total number of experiments and $T_{dof}$ represents the total degrees of freedom. $\boldsymbol{F_{a,1,fe}}$ in Equation 5 represents the 95% confidence level, the α significance level, the error degree of freedom, respectively. $V_e$ represents error variance, $\boldsymbol{n_{eff}}$ the effective iteration count and R the number of repetitions for validation experiments. The values of these parameters for confidence interval calculations are given in Table 8. As a result, it was calculated as $\boldsymbol{CI_{NC} = 0.031}$ for the number of bacterial colonies obtained experimentally.

**Table 8.** Values used in confidence interval calculations

| | ***Ve*** | **Fα,1, *fe*** | **α** | ***Fe*** | ***N*** | **R** | **$T_{dof}$** | ***Neff*** |
|---|---|---|---|---|---|---|---|---|
| Nc | 0.000134 | 5.1174 | 0.05 | 9 | 16 | 1 | 6 | 2.285 |

On the other hand, as seen in Table 5, the ideal level group for the lowest Nc is the 4th level of the current parameter and the duration parameter. Equation 6 was used to calculate the estimated optimum Nc values. The $\boldsymbol{T_{Nc}}$ value (1.5 log cfu $mL^{-1}$) is the mean of the sum of the bacterial colony counts obtained for each current and duration combination. As a result, the $Nc_{opt}$ value was calculated as 1.32 N by replacing the relevant data in Equation 6.

$$\boldsymbol{Nc_{opt} = (Current_4 - T_{Nc}) + (Duration_4 - T_{Nc}) + T_{Nc}} \quad (6)$$

When the values were applied;

$\boldsymbol{Nc_{opt} = (1.353 - 1.5) + (1.467 - 1.5) + 1.5 = 1.32}$ When the values were applied;

$\boldsymbol{[Nc_{opt} - CI_{Nc}] < Nc_{exp} < [Nc_{opt} + CI_{Nc}] = [1.32 - 0.031] < 1.31 < [1.32 + 0.031] = 1.289 < 1.31 < 1.351}$

**Table 9.** Validation tests results

| **Nc (log cfu $mL^{-1}$)** | **Current (μA)** | **Duration (s)** | **Experimental** | **Assumption** | **Error (%)** |
|---|---|---|---|---|---|
| Optimum | 20 | 60 | 1.31 | 1.297 | 0.99 |
| Random | 12 | 17 | 1.56 | 1.539 | 1.346 |

The values obtained at the optimum process parameters for the bacterial colony counts, which are the output parameters, are within the confidence interval limits. This indicates the accuracy of the optimization process (at a 95% significance level). In addition, the accuracy of the optimization process was tested at the optimum and random levels of the process parameters; the results are given in Table 9. It has been reported that the error rate between the experimental and estimated values should be less than 20% in terms of the reliability of the optimization process [32]. Examining Table 9, it can be argued that the deviation between the experimental and estimated values is within acceptable limits, and the optimization was carried out successfully.

## CONCLUSION

*Pseudomonas aeruginosa* is an undesirable bacteria presents in raw milk. It spoils the product under cold storage conditions and in heat-treated milk, thanks to its metabolic residue enzymes. In addition, the ingestion of bacteria through contamination causes severe respiratory diseases, especially in people with weakened immune systems. It is highly important to eleminate the presence of this bacteria in milk in terms of both public health and dairy products problems. The present study suggests that the silver anode technique can be used for antibacterial purposes against *Pseudomonas aeruginosa* bacteria, which is an issue in the dairy industry. The Taguchi method can be successfully applied in determining the optimum current-time relationship in the silver anode technique. According to the results of S/N analysis, the optimum process parameters for the present study's lowest bacterial colony count (Nc) were determined to be 20 µA current and 60-sec duration. The variance analysis results revealed that the most effective process parameter on the bacterial counts of the colonies was the current. It was seen that the regression equation developed to estimate the bacterial counts of the colonies yielded results very close to the experimentally obtained values. Accordingly, it is predicted that the industry will accelerate the combat against bacteria by using the regression equation developed to estimate the colony counts of *Pseudomonas aeruginosa* ATCC 27853. Therefore, the results obtained in the study are essential in this respect.

**Conflict of interest:** The authors declare no conflict of interest.
**Authors' contribution:** Sergul Cibik performed the analyses and wrote the article. Ayhan Duran directed the study.

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