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Design of fish processing equipment: exploring cleaning brush performance and material properties to minimize biofilm deposits

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Abstract

The development of bacterial biofilms in fish processing plants may facilitate the growth of human pathogenic bacteria such as *Listeria monocytogenes* and may therefore compromise food safety. In the Norwegian aquaculture industry, the cleaning of fish processing plants is the final process step during fish manufacturing processes. This article explores the efficiency of brushes of different designs in combination with using different cleaning methods in cleaning three types of material. A total of seven different brush designs (including those with soft, medium, and hard bristles; bristles also varied in length and density) and operational movements (linear versus rotational), were tested. It is believed that in the future, robots may clean fish processing plants, and equipping such robots with cleaning tools may significantly contribute to increasing food safety. The objective of verifying the performance of cleaning tools used in fish factories is to determine how effective they are in terms of the cleaning and removal of biofilm and how they perform with regard to cross-contamination. Test items of stainless steel, aluminum alloy and high-density polyethylene were experimentally inoculated with *Pseudomonas fluorescence* and *Staphylococcus aureus*, which were allowed to develop biofilms. Cleaning efficiency was analyzed spectrophotometrically by swabbing the cleaned test items and measuring optical density at 600 nm in the swab eluates after overnight incubation. Cleaned test items and uncleaned controls were also stained with SYBR Green and photographed under ultraviolet light to evaluate biofilm removal efficiency by image analysis. Cleaning trials demonstrated that a rotating brush (240 rpm) performed better than brushes using a linear movement for the removal of biofilms on all material types, and that equipping robots with such tools may significantly improve cleaning performance. It is also found, however, that rotating brushes may introduce the risk of cross contamination due to splashing of bacteria into the air. The research findings and the methodology used for verification in this paper will serve as the foundation for future research on hygienic design and cleaning performance in the global fish processing industry.

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Keywords: multi-domain design requirements; experiments; aquaculture; biofilm

1. Introduction

This article presents experiments concerning how material properties and cleaning tools can affect the efficacy of cleaning of fish processing equipment (FPE) in the Norwegian aquaculture industry. After each day of producing and processing fish, FPE must be cleaned to address the risk of bacterial contamination and ensure food safety [1,2]. A specific challenge when cleaning is contamination by the human pathogenic bacterium *Listeria monocytogenes*. This pathogen has not been fully controlled in food production [3] and is able to survive extreme environmental conditions, which makes it difficult to eliminate [4]. The cleaning of FPE is a demanding manual job that is often performed at night, and fish processing facilities (FPPs) often invest substantial amounts of resources, money and time into cleaning. The cleaning process is in addition unstable in terms of the goodness of the cleaning results [5]. Efforts have been made towards enabling robots to clean FPPs [6,7], but such work is still ongoing. In this field of research, further exploration of how robots may be used to clean FPPs and, if so, whether it would be possible to equip such robots with cleaning tools (e.g., brushes) to aid in such work is required. In addition, advancements in hygienic design and greater

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understanding of how design impacts the cleanability of FPE are required [8].

It is a working theory that the design of FPE greatly influences how easy biofilm, and consequently *L. monocytogenes*, will grow on it. This theory will be investigated by testing the efficacy of cleaning contaminated metal surfaces by rinsing them first with water alone, followed by cleaning using brushes in combination with water. Three materials are tested: AISI 304 steel, aluminum alloy 6082 and PEHD 500 High Density Polyethylene (PEHD500 in short for the remainder of this article).

Steel and thermoplastic are two very commonly used materials in the aquaculture industry due to their corrosion resistance and fine surface roughness [9,10]. The aluminum alloy in this test also has excellent corrosion resistance but a rougher surface roughness than steel and the thermoplastic [11], and is thus not commonly used in the aquaculture industry.

1.1. Scope

In total, 20 material samples—eight steel, seven aluminum and five plastic—were made (see Figure 1). They have varying geometries: Some are welded together, some are bolted together, some are bent 90 degrees and some are kept plain.

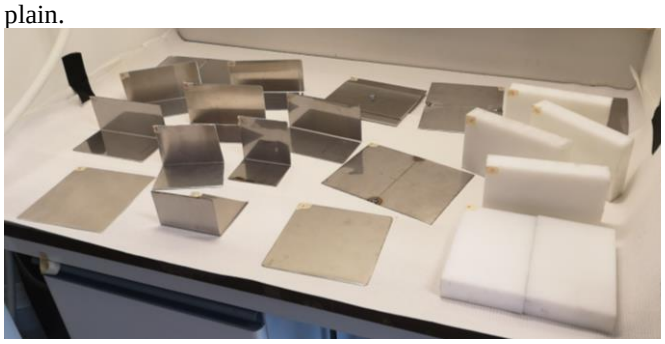

Figure 1. Material samples

Both the steel and aluminum samples are made in two sizes; 150 x 100 mm and 100 x 100 mm. The PEHD 500 is cut into 150 x 150 mm pieces. Some of the aluminum and steel 100 x 100 mm plates are welded together with a butt weld; however, a small gap is created between the plates to allow bacterial biofilm formation. The same is done for two plastic plates, but these are screwed together. In addition, two larger of the larger plate parts of steel are assembled together using a bolt and nut to investigate bacterial formation between the plates. Each sample is marked with a number for keeping track of the results.

1.2. Tools

As can be seen in Figure 2, several tools were obtained to test their efficacy in removing biofilm: 1) hard, 2) medium and 3) soft brushes. Special tools, as depicted in Figure 3, were also used: 1) pipe brushes, 2) rotational brushes and 3) rotational sponges. The rotational brushes and sponges were connected to a drill via a 3D-printed fixture.

Figure 2. Brushes

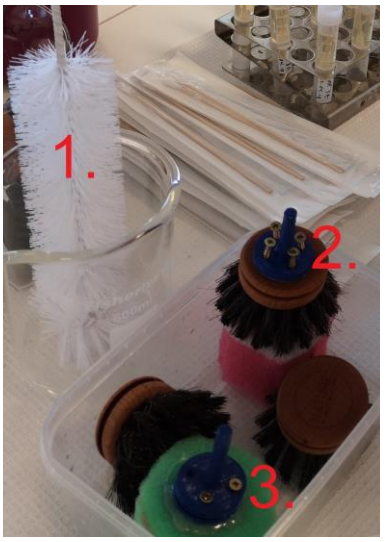

Figure 3. Rotational brushes

2. Method

2.1. Material samples

A mixture of two bacteria, *Pseudomonas fluorescens* and *Staphylococcus aureus*, was prepared. *P. fluorescens* and *S. aureus* were used instead of *L. monocytogenes* since *L. monocytogenes* is quite dangerous to work with. In addition, a nutrient broth was brewed to facilitate the growth of biofilm. A growth container was filled with *P. fluorescens* and *S. aureus* and nutrient broth; the material samples were inserted in the container and kept at 25 °C for two weeks to allow for the formation of a biofilm across the surface and the colonization of the test samples.

Each test sample was attached to a stand that held it in place over a sink. Purified water was used to rinse the samples on one side to evaluate the efficacy of water alone in removing biofilm. The samples were then turned around and cleaned with both water and tools and left to dry. An after picture was taken with the apparatus, and the before and after pictures were subsequently compared to measure the cleaning efficiency.

2.2. Brushes – cleaning and measurements

The scrubbing with brushes was done with using a linear movement at a slow speed and with little force and no sudden movements, in a manner intended to mimic that of a cleaning robot. The same pattern and movements were used for all of the different brush types, including the rotational brushes. The cleaning operation had a duration of 10 seconds, and the

mechanical rotation of the tools had an rpm of 240.

After being used for the cleaning of the samples, the tools were cleaned in a bowl of water and then left in a nutrient broth; this is done to subsequently measure the growth of bacteria on the tools. Cotton swabs were used to collect bacteria from both the tip and the core of each brush and marked according to brush type and whether they were taken from the tip or the core. Only one sample is collected from the sponge.

Two containers, one for tip samples and one for core samples, were filled with 400 µl of nutrient broth using a pipette. The cotton swabs were sealed inside the containers to allow for comparison of bacterial growth on the tips and at the cores of the brushes. The process was repeated for every cleaning tool. The cotton swabs were stored in the nutrient broth at 35 °C overnight.

A spectrophotometer was used to measure the bacteria level by reading absorbance at 600 nm (A_{600}); it was calibrated to the growth medium used, meaning that the zero point was the amount of light that penetrates the growth medium. The difference in A_{600} between the bacteria sample and the growth medium control sample was measured and was used to determine the concentration of bacteria in the sample. This approach is not accurate enough to produce quantifiable results concerning the bacteria level, but it will allow for comparisons to be made between samples.

Each sample was evacuated from the incubation container once the biofilm was formed and allowed to dry before further handling. The samples were then immersed in a SYBR Green solution diluted to 1x for approximately 1 hour before they were taken out and allowed to dry. A picture of each sample was taken using Biorad's ChemiDoc MP [12] using the SYBR Green settings before any cleaning commenced. A corresponding picture was taken after cleaning, in which the biomass will stand out as a contrast to the background as seen in Figure 4. The efficiency of the cleaning was calculated using equation (1) below, by comparing sizes of the areas with biofilm in the before and after pictures:

$$Efficiency = \frac{Area_{After}}{Area_{Before}} \quad (1)$$

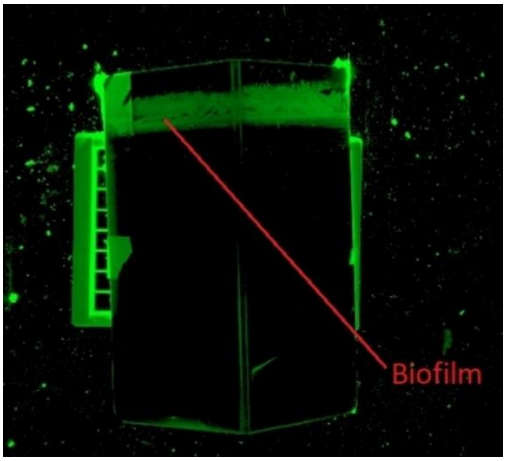

Figure 4. A sample with biofilm taken with the ChemiDoc MP system before cleaning shows biofilm formation along the top of the sample. The picture was taken with the SYBR Green settings.

The area differences that could be identified during one of the cleaning tests are illustrated in Figure 5 below, which shows the cleaning results from test sample 5.

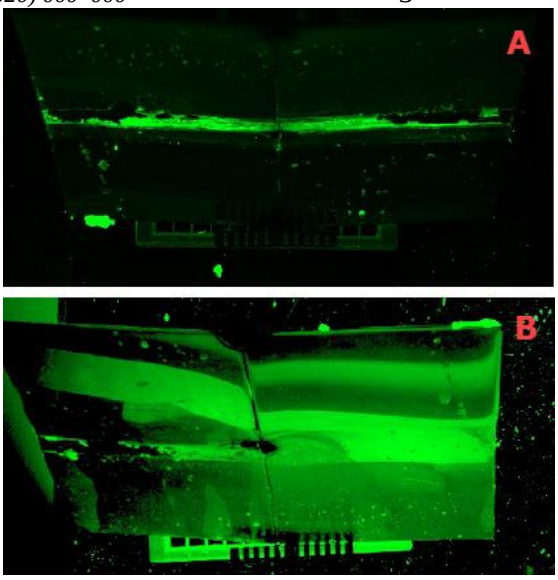

Figure 5. Test sample 5. A) Before cleaning, B) after cleaning. The left half of the surface in B) was cleaned with only water, while the right side was cleaned with tools and water.

3. Results and Discussion

The first test (rinsing with water) established how much biofilm water alone would remove. In addition, it provided insights into how the surface properties of each material impact cleaning results.

Table 1. Overview of cleaning results. Efficiency is measured for both water rinsing alone and cleaning with tools.

Test No.	Material Type	Cleaning Tool	Water test - biofilm areal			Cleaning test - biofilm areal		
			Before	After	% cleaned	Before	After	% cleaned
1	Steel	Rotating Sponge	36	30	16 %	70	36	49 %
2	Steel	Soft brush	10	8	19 %	100	6	94 %
3	Steel	Medium brush	42	31	26 %	16	1	97 %
4	Steel	Pipebrush	50	36	29 %	58	17	70 %
5	Steel	Circular brush	176	138	22 %	12	0	100 %
6	Steel	Hard brush	175	140	20 %	175	73	58 %
7	Steel	Sponge - Manual	39	32	17 %	33	5	85 %
8	Alu	Rotating Sponge	52	42	20 %	105	4	96 %
9	Alu	Soft brush	240	206	14 %	85	16	81 %
10	Alu	Medium brush	100	90	10 %	73	30	59 %
11	Alu	Pipebrush	97	70	28 %	100	4	96 %
12	Alu	Circular brush	100	85	15 %	100	2	98 %
13	Alu	Hard brush	100	95	5 %	44	4	90 %
14	Alu	Sponge - Manual	100	80	20 %	100	14	86 %
15	Plastic	Rotating Sponge	100	80	20 %	100	2	98 %
16	Plastic	Soft brush	100	95	5 %	100	5	95 %
17	Plastic	Medium brush	100	80	20 %	100	25	75 %
18	Plastic	Circular brush	100	85	15 %	100	1	99 %
19	Plastic	Hard brush	100	80	20 %	100	4	96 %
20	Steel	Medium brush	100	76	24 %	100	2	98 %
			Average cleaned		18 %	Average cleaned		86 %

As shown in Table 1, biofilm is easier to remove from steel compared to the other materials in the test. This is due to the relative higher surface smoothness of steel compared to the other materials. An estimated 50–60% of the total biofilm fell off of the steel surfaces before cleaning commenced (during drying), while, as can be seen in Table 2 below, this figure was an estimated 5% or less for the other materials.

Table 2. The average cleaning result, categorized by material type.

Total biofilm cleaned	Drying	Water test	Tool tests
Stainless steel	55 %	22 %	95 %
Aluminium	5 %	16 %	90 %
Plastic	1 %	16 %	93 %

During water rinsing, stainless steel samples performed significantly better than the other materials, with 22% of biofilm removed compared to 16%, meaning that 6% more biofilm may be removed during water rinsing by choosing stainless steel over

aluminum or plastic.

During the last tests, in which cleaning was performed with a combination of water and cleaning tools, stainless steel performed best, with 95% of the biofilm being removed. For aluminum and PEHD500, the removal rates were 90% and 93%, respectively, as can be seen in Table 2.

A total of seven tool designs were tested, of which four required manual scrubbing and three were used in mechanical rotational movements. The tools all varied in material, length and density. The results of the cleaning tests with tools can be seen in Table 1; furthermore, in Table 3, they are grouped into three distinct categories: tool type, movement type and material type.

Table 3. The results of the cleaning tool test categorized by type, movement and material types

Average cleaning by tool	Percentage
Rotating Sponge	97 %
Soft brush	90 %
Medium brush	90 %
Pipebrush	83 %
Circular brush	99 %
Hard brush	93 %
Sponge - Manual	85 %
Average cleaning by movement	
Linear robot movement	90 %
Rotation	93 %
Average cleaning by material type	
Sponge	91 %
Soft	90 %
Medium	94 %
Hard	90 %

The circular brush was the top performer with regards to cleaning, as it removed 99% of the biofilm, while the pipe brush exhibited the worst performance. This poor performance was due to, among other reasons, the mess that the brush made during cleaning; this resulted in the cleaning test with this brush being terminated prematurely, as biofilm was splashed into the air and thus posed the risk of contamination.

3.1. Brush contamination

Brushes introduce the risk of cross-contamination. The cross-contamination potential of these brushes was evaluated by measuring the number of bacteria left on them after rinsing. The results of the cleaning test displayed a clear trend in terms of how the bacteria colonized the tools. The first observation was that there was a higher density of bacteria at the core of each tool than at the tip (Table 4). This finding can be explained with reference to the increased difficulty of cleaning the core of the tool when compared to the tip due to the fact that the core is less exposed.

Table 4 Absorption values from spectrophotometry. Higher values equal higher density of bacteria.

Sample name	Sample No.	Abs	Amount compared to average
Soft brush - Bottom	1	0,573	113 %
Soft brush - Top	2	0,577	114 %
Medium brush - Bottom	3	0,71	140 %
Medium brush - Top	4	0,61	121 %
Hard brush - Bottom	5	0,578	114 %
Hard brush - Top	6	0,368	73 %
Pipebrush - Bottom	7	0,249	49 %
Pipebrush - Top	8	0,225	45 %
Circular brush - Bottom	9	0,596	118 %
Circular brush - Top	10	0,561	111 %
Sponge - Surface	11	0,513	101 %
Average	-	0,505	

The pipe brush had fewer bacteria due to its bristles, which were hard and fewer in number compared to the other brushes, thus offering less of an area for the bacteria to attach to and grow on and making this brush easier to clean. The medium brush had the highest bacteria density. This brush had bristles of a shorter length and a much higher density/number of bristles, which provided an ideal habitat for the bacteria to grow in.

On average, the bacteria density at the core was 18% higher when compared to the tip. The brush with hard bristles had as much as 57% more bacteria at the tip, with that with bristles of medium hardness had 16% more, and the soft brush had 1%, as can be seen in Table 5 below.

Table 5 Percentage difference been bacteria density at the core and tip of each cleaning tool.

Sample name	Difference in percentage
Soft brush	-1 %
Medium brush	16 %
Hard brush	57 %
Pipebrush	11 %
Circular brush	6 %
Average	18 %

It is currently unclear as to why the soft brush had less bacteria at its core when compared to its tip, but this finding could be the result of measurement or other human errors.

A grading system was created to summarize the capabilities of the different brush types (Table 6). Two tools, namely the circular brush and the rotating sponge, both of which were attached to a drill, scored highest in the removal of biofilm. For the bacteria value measured on the brushes after cleaning, the performance was generally low, with the hard brush and pipe brush scoring best. Overall, the two tools with rotational cleaning scored highest when combing their scores, indicating that a rotating device may be beneficial in cleaning biofilm.

A cleaning tool intended for use with robots should thus feature the following attributes:

- Effectiveness
- Adaptability
- Easy maintenance

Table 6 Performance score for cleaning (biofilm removal), performance score of each brush (based on A_{600}) and total score.

Performance score	Grade	Ciruclar brush	Soft brush	Medium brush	Hard brush	Pipe brush	Sponge	Rotating sponge
<80%	1							
80-85%	2							
85-90%	3							
90-95%	4							
95-100%	5							

Bacteria level	Grade	Ciruclar brush	Soft brush	Medium brush	Hard brush	Pipe brush	Sponge	Rotating sponge
800	1							
750								
700								
650	2							
600								
550								
500								
450	3							
400								
350								
300								
250	4							
200								
150	5							
0								

Total score	Grade	Ciruclar brush	Soft brush	Medium brush	Hard brush	Pipe brush	Sponge	Rotating sponge
1	E							
2								
3	D							
4								
5	C							
6								
7	B							
8								
9	A							
10								

The *effectiveness* attribute describes a tool's ability to produce the desired cleaning result and is measured with reference to the percentage of the sample surface cleaned. The adaptability attribute indicates how well a tool adapts to surfaces, shapes and obstacles (e.g., by being jointed or offering an expandable reach or other features that would increase the likelihood of use). The last attribute, *maintenance*, refers to the deterioration and wear of a tool and to its inherent ability to avoid cross-contamination and ease of cleaning (decontaminated). An example of a feature related to this attribute would be a self-flushing system.

The sponges used in this work are conventional kitchen sponges found in most households. Such sponges, however, are known to harbor large amounts of bacteria [13]. Cardinale et al. found that a well-maintained sponge that was frequently cleaned had a significant prevalence of 2 of the 10 most dominant bacteria; thus, this sponge had a higher pathogenic potential when compared to a dirty sponge. Therefore, the use of sponges for cleaning FPPs must be carefully considered, and a sponge-based cleaning tool should have rigorous cleaning and decontamination protocols and a design that makes sanitation straightforward. In order to reduce cross-contamination, it is recommended that both brushes and sponges be stored in disinfectant when not in use and/or rinsed thoroughly prior to their next use.

4. Conclusions and Further Work

This research investigated how the properties of three materials, two common and one uncommon, used in FPPs affect the growth of biofilm on those materials. This was investigated through cleaning experiments in which bacteria were grown on material samples and cleaned off using two methods: 1) rinsing with water and 2) using brushes. It is concluded that stainless steel is the preferred material for use in FPPs due to the limited amounts of bacteria that is able to grow on this material when compared to PEHD500 and aluminum.

Through testing bacteria levels in the brushes used during cleaning and measuring their cleaning abilities, it was further found that cleaning using rotational brushes is the preferred method. A supplementary remark regarding brushes is that brushes with very hard bristles introduce a substantial risk of cross-contamination.

Further work is needed to investigate how brushes and tools should be implemented and used for cleaning in FPPs, possibly alongside with robotic cleaning. In addition, future studies should investigate how brushes will impact cleaning effectiveness over time and how they may be sanitized effectively.

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