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Wood in Food - Measuring Methods

Partial report 1

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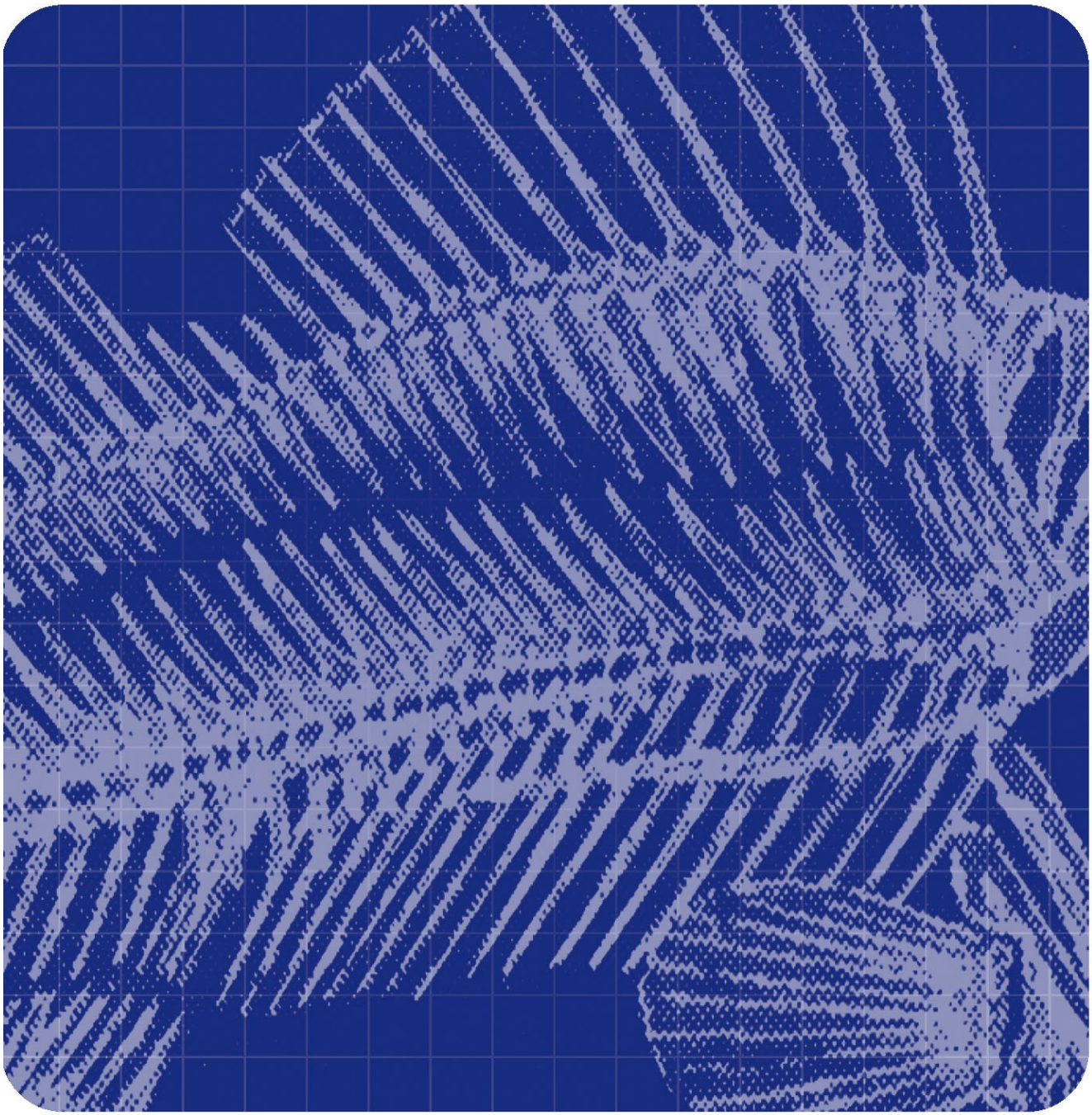

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Summary:

The aim of this project is to develop and evaluate measuring methods to control the hygienic status of wood in food industry. This is a part of the project “wood in the food industry” where the suitability of wood products used in the food industry is studied. The Nordic Wood programme funds the project with participants from Iceland, Denmark, Sweden and Norway. The development and evaluation of measuring methods is a co-operation between the Icelandic Fisheries Laboratories and the Norwegian Institute of Fisheries and Aquaculture Ltd. We have tested five different measuring methods; contact method, soaking of sample in water, scraping, swabbing and liquid media poured on the surface. We used *Halobacterium salinarum* and *Pseudomonas sp*. as test organisms. None of the methods gives optimum results, but among the five methods, we recommend the contact and the swabbing as the most convenient and suitable measuring methods to be used in the industry. The contact method is easy to perform and convenient for a screening of the hygienic conditions of the wood. The swabbing method is easy to perform, quantitative, not destructive and applicable on all kinds of surfaces.

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1 Introduction

The aim of this work is to develop and evaluate measuring methods to control the hygienic status of wood in food industry.

When developing a microbial test method there are some general requirements to fulfil; easy to perform, cheap, safe, secure, fast and not labour consuming.

All these steps have been considered in this project (Lorentzen, 1999) and (Guðbjörnsdóttir, 1999). The experiments are based on traditional test methods, which involve 3-6 days before any result is available. A test method consists of two steps; sampling (step 1) and analysis (step 2). Step 1 must be easy to perform, and should not require any special knowledge of microbiology. To perform the analysis (step 2), there are two options. One; the analysis is performed in the plant, or two; the analysis is performed in an independent laboratory. Where to perform the analysis must be considered in each case depending on location, access to laboratory facilities, knowledge etc.

In this experiment we have tried out new softwood which is common in pallets. Although wood is not permitted in the food industry, some plants still use it (e.g. saltfish industry). In addition, experiments of plastic and stainless steel have been performed to compare with wooden samples. Scanning Electronic Microscopy (SEM) was used to evaluate the adherence of bacteria on surfaces used in this experiment.

These experiments have been performed in close collaboration between the Norwegian Institute of Fisheries and Aquaculture Ltd in Tromsø (FF) and the Icelandic Fisheries Laboratories in Reykjavik (IFL).

Both laboratories have tried out five different measuring methods. At FF there have been done experiments on halophilic bacteria; *Halobacterium salinarum*. IFL have been done experiments on *Pseudomonas* sp. isolated from fish processing environment.

2 Materials and methods

2.1 Wooden samples

The test specimens from Høylandet Treindustri A/S were sampled from the normal raw material; soft wood, for their pallet production. Spruce (*Picea abies*), was chosen to be used in the experiment. Boards of dimension 19x100 mm, of good quality and length more than 5 m were chosen.

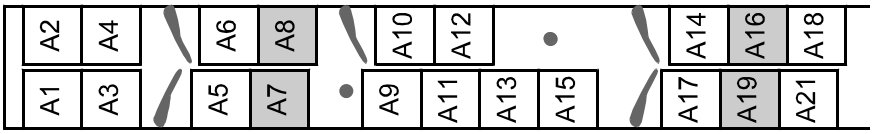

Figure 1. Schematic drawing of the board

Figure 1 shows a schematic drawing of the board. The boards numbered A-H were split in two along the centre. Samples approximating the size 50x50 mm were cut and marked as shown in the figure above. Larger knots and other impurities were deleted. For every meter, starting one meter from the leading end of the board, two paired samples were taken out for density and water content determination.

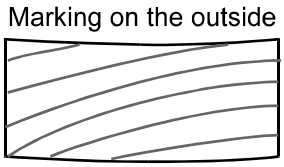

Figure 2. End surface of the wooden sample. Each sample was marked with a code on the outside.

Figure 2 show the end surface of the wooden samples. The annual ring pattern shows the outside and pith side of the board. All markings were done on the outside, as the pallet wood manufacturer generally prefers that the pith-side of the board is up in the finished products. The reason is that when drying, the board will cup, tending for the annual rings to straighten. The pith-side of the board is normally being slightly convex as indicated in the figure.

In the experiments we have used both dry and wet wooden samples. The dry samples were put in the lab a couple of days prior to the experiments. We made the wet samples by soaking the wooden samples into water for 18 – 20 hrs just before the experiment started. It was very important to make sure that all the wooden samples were completely covered with water.

Samples of plastic (polyethylene) and stainless steel (AISI-304), were tested to compare with results from the wooden samples.

2.2 Chemical and physical measurements

In addition to the microbial tests, chemical and physical measures were carried out. Density, water content and water activity (aw) was estimated in the wooden samples made for the experiments. These factors are believed to influence the survival and growth of the microorganisms in wood.

2.2.1 Density

The density is measured by first measuring the size of the sample by a slide calliper, and then the samples are weighed. The density is found by dividing the weight by the volume. This density is the so-called density at current moisture content (here: u = 12-14%). The basic density is also measured by dividing the weight of the dried samples by the volume (volume at actual moisture content).

The density of wood may differ a lot within one board. According to the literature the average density of spruce (*Picea abies*) is 470 kg/m^3, but because of the non-homogenous nature of wood, it may vary from 330 kg/m^3 to 680 kg/m^3.

2.2.2 Water content and water activity.

The initial water content in each sample was measured in the beginning. The water content was from 12.4 -14.4 % water. The samples were kept in the lab for at least 4 days or until they stopped loosing weight. All samples were weighted before tested and the wet samples were weighted again after soaking in water. The gain of weight during soaking was estimated to approximately 60 %. The final water content for the wet samples were 35-40%. At IFL the water-activity was measured with a_w Wert Messer meter (Durotherm) on selected samples of wood, both dry and wet, before and after different contamination time. The water activity was measured at ambient temperature. The growth of microorganisms demands the presence of water in an available form. It is generally accepted that the water requirements of microorganisms should be described in terms of the water activity (a_w) in the environment. This parameter is defined by the ratio of the water vapour pressure of the sample to the vapour pressure of pure water at the same temperature $a_w = p/p_o$. The minimum values reported for growth of some microorganisms with respect to water activity is shown in table1.

Table 1. Minimum levels of water activity (a_w) permitting growth of some micro-organisms at optimal temperature.

Microorganism	**a_w**
Bacteria	0.91
Yeast	0.85
Moulds	0.80
Halophilic microorganism	0.75
Xerophilic moulds	0.65
Osmophilic yeast	0.60

2.2.3 pH -Values

The pH-value in the wood may influence the growth and survival of microorganisms added on the surface. Investigation has shown the internal pH of the cells to be affected by the pH of the environment (Silliker et al, 1980). Many microorganisms can grow well between pH 5-8. In the literature, the pH value in spruce (*Picea abies*) is 5.3 (Fengel, and Wegener, 1984). The pH of the wooden samples was not measured in the experiments.

2.3 Bacterial strains

H. salinarum and *Pseudomo*nas sp. were used to analyse the effectiveness of chosen measuring methods.

H. salinarum

This microorganism can be a problem in the salt-fish industry. The halophilic bacteria are the most common cause of "pink" (pink spots) on salted fish.

When grown under optimum conditions the *H. salinarum* may be rod or disc shaped. Some strains are highly pleomorphic even under optimum growth conditions. Most strains are strict aerobes, but facultative anaerobes growing with or without nitrate have been described in the literature (Larsen,1984). The optimum temperature is 40 °C, no growth occurs below 7-8 °C. The halophilic microorganisms are able to survive up to 82 °C (van Klavern and Legendre, 1965). Colonies are pink, red, or red-orange, and are opaque to translucent and oxidase- and catalase- positive. Most isolates require at least 2.5 M (15 %) NaCl and 0,1 – 0,5 M Mg^{2+} for growth . They grow best in 3,5 – 4,5 M (20 – 26 %) NaCl, and also grow well in saturated NaCl solution (>5 M, or > 29 % NaCl) (Larsen, 1984).

Growth is relatively slow; generation times of 3-6 hrs are the fastest that have been reported in laboratory experiments.

Pseudomonas sp.

Pseudomonas sp. isolated from fish processing environment where used for the experiment at IFL. Microorganisms are found in substantial numbers on the skin, gill and in the intestine of live fish. The numbers and types of bacteria present are related to the environment in which the fish are caught. *Pseudomonas* sp. among other bacteria are detected on fish caught in temperate countries and can take part in the spoilage pattern. Some *Pseudomonas* sp. are also known for producing polysaccharide filaments, which enhance their attachment to surfaces in contact with food.

Minimum generation time for *Pseudomonas* sp. have been reported as 1 hour in laboratory experiments (Nickerson, 1972).

2.4 Media

The microorganisms were detected by using specific media. To detect *H. salinarum*, we used a specific medium for halophilic microorganisms. The recipe is described in appendix no 1.

The inoculum used to contaminate wooden samples with *H. salinarum* had been growing for 3-5 days in a liquid media (broth). The microorganisms had optimum conditions; 25 % NaCl, at 37 °C, light, aerobic condition and continuous shaking. The final cell concentration before contamination varied between 10^7 - 10^8 colony forming units pr ml (CFU/ml).

To detect *Pseudomonas* sp. we used a plate count agar (PCA-Difco) with 0.5 % NaCl added and brain heart infusion (BHI-Difco). The inoculum used to contaminate wooden samples with *Pseudomonas* sp. had been grown in a liquid media (broth) for 3 days. The microorganism was incubated at 22°C. The final cell concentration was 10^7-10^9 CFU/ml before contamination.

When preparing a contamination for the wooden samples, we also used fish juice. Fish juice is made of fish and contains nutrition that the microorganisms are exposed to in the fish industry. To simulate this, we performed parallel tests. First, we made a contamination containing the microorganism and broth. Secondly, we made a contamination containing the microorganism and fish juice. The fish juice used for *H. salinarum* was corrected for the content of salt. The recipe for making fish juice is shown in appendix no. 2.

2.5 Disinfection of samples

To avoid any contamination from the wood, the samples were disinfected prior to the experiments. Disinfection was only carried out for the experiments with *Pseudomonas* sp. as contaminants. The wooden samples contaminated with *H. salinarum* were not disinfected because of very strict growth conditions; requirements for high levels of NaCl.

Samples of wood, plastic and stainless steel were sterilised in an autoclave at 121 °C for 15 minutes. Before putting them into the autoclave, the samples were wrapped in aluminium paper and put in autoclaveable bags, sealed with an autoclaveable tape.

2.6 Contamination

A volume of 0.5 ml of the inoculum was spread evenly on the pith side of the wooden sample surface with the side of the pipette or z-shaped rod. The same volume was spread evenly on the plastic and stainless steel samples.

2.7 Experimental structure

Table no 2 shows the structure of the experiments carried out at IFL and FF. In the first experiment (no 1), 5 different measuring methods were tried out with different strains and with the same sampling intervals. The contamination levels for the microorganisms were relatively high ($10^6 – 10^9$ CFU/ml). This was done in order to have a sufficient concentration to be able to recover the bacteria after contamination. This was also done to simulate an extremely high contamination. Some of the measuring methods were also tried out on samples of plastic and stainless steel. In the second experiment (no 2), only three measuring methods were carried out with different level of contamination and longer sampling intervals.

Table no 2. Structure of the experiments carried out at IFL and FF.

Experiment	**Measuring method (no)**	**Sampling intervals (min)**	**Strains / level of contamination (CFU/ml)**	**Institute**
1a) Measuring methods- wood	1, 2, 3, 4, 5	5, 30, 120, 960	*Pseudomonas* sp. / 10^9 *H. salinarum* / 10^7 - 10^8	IFL FF
1 b) Measuring methods – plastic	1, 2, 3	5, 30, 120, 960	*Pseudomonas* sp. / 10^7 –10^9	IFL
1 c) Measuring methods – stainless steel	3	120, 960	*Pseudomonas* sp. / 10^7 –10^9	IFL
2. Different levels of contamination	1, 3, 4	30, 120, 960, 7200	*Pseudomonas* sp. /10^3 – 10^9	IFL
	3, 4	5, 120, 960, 7200	*H. salinarum* /10^5 – 10^8	FF

For further information, test plans for FF and IFL are shown in appendix no 3 and 4.

2.7.1 Measuring methods

In the first experiment (1 a), five different measuring methods for recovery of the bacteria from the contaminated wooden samples were studied. The choice of methods is based on an article (Ak. et al, 1993) and a review written by H. Lauzon (Lauzon, 1998). After performing the methods, the petri plates were incubated at the optimum growth conditions for the test organism. Samples containing *H. salinarum* were incubated at 37 °C, under light and aerobic condition. Samples containing *Pseudomonas* sp. were incubated at 22°C. Samples were contaminated for 5, 30, 120 and 960 minutes and all samples were duplicates.

In the first experiment (1 b), measuring method 1 – 3 were tested on samples of plastic and method 3 on stainless steel (1 c). Tests on plastic and stainless steel were only done for *Pseudomonas* sp. All measuring methods are shown in photos in appendix no 5.

Method no 1

After contamination, the wooden samples were put on a surface of nutrient agar in a petri plate for 2 minutes. The petri plate was put in a plastic bag to keep the samples from drying out.

Method no 2

After contamination, the bacteria were recovered by soaking the contaminated surface in a liquid of sterile peptone/salt water solution in a petri plate. The wooden sample was put in the liquid for 1 minute while shaking. The numbers of microbes in the salt/water liquid was determined by plate counting.

Method no 3

After contamination, we swabbed the surface by using a sterile cotton-wool (swab). Before swabbing, the swab was dipped into a sterile peptone / salt water liquid. The swab was put on the contaminated surface, and stroked over according to a defined pattern. Afterwards, the swab was stirred in the sterile peptone / salt water liquid. The numbers of microbes in the salt/water liquid was determined by plate counting. The swab used by IFL was made of hydrophobic cotton. Comparison tests between swabs used by FF and IFL showed no difference.

Method no 4

After contamination, the surface layer of the wooden sample was removed by scraping with a sterile scalpel. The amount of splinters was determined by weight. The splinters were put in a tube containing sterile peptone/salt water liquid and stirred. The numbers of microbes in the salt/water liquid was determined by plate counting.

Method no 5

After contamination, we added melted agar over the surface of the wooden sample. The agar was left on during incubation. To avoid the samples to dry out, we put them in a container / plastic bag that was not sealed.

2.7.2 Different levels of contamination

In this experiment, the methods no 1, 3 and 4 were repeated (experiment no 2). Different levels of contamination and longer intervals of incubation were also tested in order to obtain conditions similar to the industry.

2.7.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to evaluate the adherence of bacteria on surfaces used in this experiment. The bacteria were inoculated onto the surfaces of wet and dried wood, plastic board and stainless steel. The tested inoculate were both with high and low number of bacteria. The adherence of bacteria on wood after different contamination time was evaluated.

Conventional chemical preparation methods were applied to study the adherence of bacteria on surfaces used in this experiment. All such methods involve three distinct processes of chemical fixation, solvent dehydration and then the removal of the dehydration solvent ("drying").

Samples for the SEM preparation were cut into small pieces by a scalpel. In comparison we used plastic (polyethylene) and stainless steel (AISI-304) that is commonly used in the food industry (tubs, pallets, surface material that become in direct contact with food). The procedure and schedule for preparation for the SEM analysis is shown in appendix no 6.

3 Results and discussion

3.1 Chemical and physical tests of wooden samples

3.1.1 Water activity

The water activity was measured at ambient temperature.

The water activity of dry samples was estimated to 0.4 - 0.5 at ambient temperature, the water content were from 10-12%. Wet wooden samples were prepared by soaking in water. The gain of weight during soaking was estimated 60 %. The water activity in wet samples was 0.95-98 with water content from 35-45%.

3.1.2 Density

It could be expected that the difference in density of a random board would be greater than it was in our samples. The samples had no knots and little of defects in the wood, compared to what is expected to find in an average board. The results of the density are shown in appendix no 7.

3.2 Microbiological tests

3.2.1 Measuring methods

All 5 measuring methods were tried out on *H. salinarum* and *Pseudomonas* sp. After performing the measuring methods, the petri plates were incubated under optimum conditions. Samples containing *H. salinarum* were incubated 4 – 6 days. Samples containing *Pseudomonas* sp. were incubated 3 - 5 days. Table no 3 shows the results of method no 1.

Table 3. Growth of H. salinarum and Pseudomonas sp. *according to method no 1; wooden sample pressed directly on the growth media (agar). Number of + indicates the intensity of growth.*

Time after contamination (min)	5	30	120	960
Dry wooden samples				
Juice				
H. salinarum (10^9)	++++	++++	++	++
Pseudomonas sp. (10^7)	++++	++++	+++	+++
Broth				
H. salinarum (10^9)	++++	++++	+	(+)
Pseudomonas sp. (10^8)	++++	++++	+++	+++
Wet wooden samples				
Juice				
H. salinarum(10^7)	+++	++	+	(+)
Pseudomonas sp. (10^7)	+++	+++	++	++
Broth				
H. salinarum (10^9)	+++	++	+	(+)
Pseudomonas sp. (10^6)	++++	++++	+++	+++

Table no 3 shows the results by using measuring method no 1; wooden sample pressed directly on the growth media (agar). The tests with *H. salinarum* show no difference between juice (fish juice) and broth (DSMZ 97, no agar). The experiments show that *H. salinarum* has a more extensive growth on dry wooden samples compared to wet wooden samples. This might be due to the differences in number of microorganisms (CFU/ml) in the contamination. We observed an uneven growth on the wet samples compared to the dry samples. On the wet samples, the growth was located where the contamination had been distributed.

For *Pseudomonas* sp. no differences were observed between juice and broth for the dry sample. For the wet samples we observed more suppressed growth of bacteria inoculated in juice compared to broth. That might be because of the material in juice could support attachment of the bacteria to the surface known as a biofilm. Biofilm is a community of microbes embedded in organic polymer matrix adhering to a solid surface, which is in contact with food (Kumar and Anand 1998). In food processing environment, bacteria along with other organic and inorganic molecules like proteins from fish and meat become adsorbed to the surface. The results from the second method are shown in table no 4.

*Table 4. Recovery (%) of H. salinarum and Pseudomonas sp. according to method no 2; soaking the wooden sample in a liquid solution containing sterile salt a*nd */ peptone water.*

Time after contamination (min)	5	30	120	960
Dry wooden samples				
Juice				
H. salinarum (10^8)	tntc	tntc	<0,01	<0,01
Pseudomonas sp. (10^{6-7})	2,18	0,19	3,53	<0,01
Broth				
H. salinarum (10^9)	0,09	0,02	<0,01	<0,01
Pseudomonas sp. (10^{7-9})	9,94	15,02	0,04	<0,01
Wet wooden samples				
Juice				
H. salinarum (10^7)	0,10	0,02	0,01	nd
Pseudomonas sp.(10^7)	5,55	9,75	1,96	11.05
Broth				
H. salinarum (10^9)	1,60	1,20	0,05	<0,01
Pseudomonas sp. (10^7)	14,23	4,64	3,94	52,86

tntc Too numerous to count
nd Not detected

Table no 4 shows the results by using method no 2; soaking the wooden sample in a liquid solution containing sterile salt and peptone water. In the experiments with *H. salinarum*, we observed low values for recovery. Based on the results in table no 4, it seems that the recovery (%) is dependent of the contamination; less recovery is due to longer time of contamination prior to sampling. The recovery of *Pseudomonas* sp. was higher from wet wood compared to dried wood. After 16 hours of contamination on wet wood we observed a higher recovery compared to shorter time intervals. In general, method no 2 is difficult to perform on a cutting board or a pallet because it is a destructive method. This method requires that we cut the sample into pieces prior sampling. The results from method no 3 is shown in table no 5.

Table 5. Recovery (%) of H. salinarum and Pseudomonas sp. according to method no 3; swabbing.

Time after contamination (min)	5	30	120	960
Dry wooden samples				
Juice				
H. salinarum (10^8)	tntc	<0,01	<0,01	<0,01
Pseudomonas sp. (10^{6-7})	na	na	na	na
Broth				
H. salinarum (10^9)	0,03	0,01	<0,01	<0,01
Pseudomonas sp. (10^{7-9})	10,67	7,59	0,02	0,02
Wet wooden samples				
Juice				
H. salinarum (10^7)	<0,01	<0,01	<0,01	nd
Pseudomonas sp. (10^7)	8,35	2,30	0,76	19,92
Broth				
H. salinarum (10^9)	0,10	0,09	0,02	nd
Pseudomonas sp. (10^7)	19,77	13,50	5,09	17,08

tntc Too numerous to count
nd Not detected
na Not analysed

Table no 5 shows the results from method no 3; swabbing the wooden surface with a cotton swab. There is no difference between wet and dry samples in the recovery. In some experiments, the recovery is quite low with *H. salinarum* compared to *Pseudomonas* sp. Differences in recovery might indicate different procedures for sampling, or other factors. The swab used at IFL is covered with hydrophobic cotton and that was believed to give more recovery but when compared, the differences did not seem to be relevant. The recovery of *Pseudomonas* sp. was higher in wet wood compared to dried wood. After 16 hours of contamination, we observed a higher recovery compared to shorter time intervals. In table no 6, the results from method no 4 are shown.

Table 6. Recovery (%) of H. salinarum and Pseudomonas sp. according to method no 4; scraping. The results are corrected according to the weight of the sample.

Time after contamination (min)	5	30	120	960
Dry wooden samples				
Juice				
H. salinarum (10^8)	tntc	tntc	0,02	0,01
Pseudomonas sp.(10^{6-7})	2,23	0,43	0,04	0,01
Broth				
H. salinarum (10^9)	7,50	0,50	0,05	nd
Pseudomonas sp.(10^{7-9})	17,12	21,62	0,13	0,36
Wet wooden samples				
Juice				
H. salinarum (10^7)	3,80	0,60	0,02	nd
Pseudomonas sp.(10^7)	4,58	7,01	8,35	6,47
Broth				
H. salinarum (10^9)	7,40	3,40	0,60	nd
Pseudomonas sp. (10^7)	17,59	6,04	5,96	52,59

tntc Too numerous to count
nd Not detected

Table no 6 shows the results from method no 4; scraping with a sterile scalpel on the wooden surface. There are differences in recovery between wet and dry samples. After a longer time of contamination, the recovery was more from wet samples compared to the dry samples after similar contamination time. In general, the recovery drops significantly between 5 and 30 minutes contamination prior to sampling except for the recovery of *Pseudomonas sp.* from wet samples. The differences in recovery between the samples might be caused by individual ways of performing the scraping. As shown in SEM photos (appendix no 8), the microorganisms are gathered in clusters; an uneven distribution of the contamination on the surface. When we do the scraping, the contamination can be removed from these clusters or from an area with no clusters. Therefore, the sampling procedure can affect the result from all tested methods.

The results from method no 5 are shown in table no 7.

Table 7. Growth of H. salinarum and Pseudomonas sp. according to method no 5; liquid agar on the surface of the contaminated wooden sample.

Time after contamination (min)	5	30	120	960
Dry wooden samples				
Juice				
H. salinarum (10^7)	nd	nd	nd	nd
Pseudomonas sp. (10^{6-7})	nd	nd	nd	nd
Broth				
H. salinarum (10^7)	nd	nd	nd	nd
Pseudomonas sp. (10^{7-9})	nd	nd	nd	nd
Wet wooden samples				
Juice				
H. salinarum(10^7)	nd	nd	nd	nd
Pseudomonas sp. (10^7)	nd	nd	nd	nd
Broth				
H. salinarum (10^7)	nd	nd	nd	nd
Pseudomonas sp.(10^7)	nd	nd	nd	nd

nd Not detected

Table no 7 shows the results from measuring method no 5; putting liquid agar on the contaminated wooden sample. We could not observe microbial growth in any sample. On samples contaminated with *H. salinarum*, we could not observe the agar on the surface of wood. We consider that the agar might have soaked into the wood.

By pouring agar over the sample some of the oxygen is excluded from the bacteria and the condition will be anaerobic. *H. salinarum* and *Pseudomonas* sp. are aerobic; they require oxygen to survive and grow. The temperature of the agar poured over the samples is about 45°C and might effect the survival or growth of the test organism. The bacteria may be damaged because of some effect from the wood and therefore more sensitive to the heat. All these factors may have influenced our results.

Experiments with *H. salinarum* and *Pseudomonas* sp. show growth with all measuring methods, except method no 5. Based on the practical experience and our results, we consider method no 1 and no 3 to be the most convenient methods to be performed in the industry. When making an interpretation of our results it is of importance to be aware of the number of microorganisms (CFU/ml) we have used in this experiment are quite higher compared to the situation in the fish industry. This was done, in order to be able to make an estimate of the recovery. In later experiments, both institutes lowered the number of micro-organisms and prolonged the time of contamination prior sampling. In addition, IFL

carried out some experiments with recovery *Pseudomonas* sp. samples of plastic and stainless steel. Method no 1, 2 and 3 was tried out on plastic samples and method no 3 on stainless steel. The results are shown in table 8 and 9.

Table 8. Intensity of growth (+) and recovery (%) of Pseudomonas sp. on samples of plastic, according to method no 1, 2 and 3. The number of "+" indicates the intensity of the growth.

Time after contamination (min)	5	30	120	960	7200
Method no 1 (contact method)					
Pseudomonas sp.(juice)	++++	++++	++++	++++	++++
Pseudomonas sp.(broth)	++++	++++	++++	++++	++++
Method no 2 (soaking of sample in water)					
Pseudomonas sp.(juice) (10^9)	4,02	5,01	0,03	0,18	na
Pseudomonas sp.(broth) (10^9)	10,20	11,80	2,30	1,40	na
Method no 3 (swabbing)					
Pseudomonas sp.(juice) (10^9)	8,48	8,17	0,38	0,07	0,18
Pseudomonas sp.(broth) (10^9)	11,00	10,70	4,20	<0,01	<0,01

na - Not analysed
Remarks: Four "+" indicates an extensive growth of microorganisms.

Table no 8 shows the results by using measuring method no 1,2 and 3. The recovery (%) of *Pseudomonas* sp. by method 1 was not evaluated because the number of bacteria were too numerous to count. No differences were observed between methods no 2 and no 3, but differences were observed when comparing the fish juice and broth contamination. It seems like the juice promotes the attachment of bacteria to the surface so they are not that easily removed from the surface. These differences were not that obvious when testing wood samples, the test results were more variable.

Table 9. Intensity of growth and recovery (%) of Pseudomonas sp. on samples of stainless steel, according to method no 3.

Time after contamination (min)	30	120	960	7200
Method no 3	25,18	26,21	0,71	0,08

Table 9 shows that after 30 minutes and 120 minutes contamination time, the recovery was rather high from stainless steel compared to the recovery from wood. The nature of the surfaces seems to affect the recovery of bacteria from different surface after short contamination time. A higher recovery from less porous material. But from this study, the prolonged contamination time did affect the recovery from stainless steel similar to the wood and plastic samples. Other authors who have stated that the number of microorganisms recovered decreases with time (Carpentier, 1997) support this. Are the bacteria waiting for an opportunity to emerge towards the surface and then contaminate the food?

3.2.2 Different levels of contamination

Different levels of contamination were tested out in order to detect the minimum level for recovery. Compared to previous experiments, the time of contamination was prolonged and the concentration of the test organism was lowered. Different levels of contamination were all carried out on samples of wood.

Table 10 and 11 show the results from experiments with method no 3; swabbing and 4; scraping. *H. salinarum* is the test organism.

Table 10. Recovery (%) of H. salinarum according to method no 3; swabbing

Minutes after contamination (min) 5 Dry / wet (d/w)	(d/w)	120 (d/w)	960 (d/w)	7200 (d/w)
5,8 x 10^8 CFU/ml	19,21 / 3,09	<0,01 / nd	<0,01 /<0,01	nd / nd
5,8 x 10^7 CFU/ml	tntc/ tntc	0,01/ 0,01	<0,01 /nd	nd / nd
5,8 x 10^6 CFU/ml	3,90 / 4,29	0,01 / 0,01	<0,01/nd	nd / nd
5,8 x 10^5 CFU/ml	9,55 / 3,81	0,04 / <0,01	nd/nd	nd / nd

tntc Too numerous to count
nd Not detected

Table 11. Recovery (%) of H. salinarum according to method no 4; scraping

Minutes after contamination (min) 5 Dry / wet (d/w)	(d/w)	120 (d/w)	960 (d/w)	7200 (d/w)
5,8 x 10^8 CFU/ml	4,65 / <0,01	4,18 / <0,01	<0,01 /< 0,01	nd/nd
5,8 x 10^7 CFU/ml	tntc / 0,20	0,06 / 0,33	<0,01 /< 0,01	nd/nd
5,8 x 10^6 CFU/ml	1,52 / 4,77	0,02 / 0,26	nd/nd	nd/nd
5,8 x 10^5 CFU/ml	5,04 / 5, 16	0,78 / 0,01	nd/nd	nd/nd

tntc Too numerous to count
nd Not detected

Table 12, 13 and 14 show the results from experiments with method no.1:contact method, 3: swabbing method and 4; scraping method. *Pseudomonas* sp. is the test organism.

Table 12. Recovery (%) of Pseudomonas sp. according to method no 1; contact method

Minutes after contamination (min) 30 Dry / wet (d/w)	(d/w)	120 (d/w)	960 (d/w)	7200 (d/w)
1 x 10^8 CFU/ml	na/++++	na/++++	++++/na	<0,01/<0,01
1 x 10^7CFU/ml	++++/++++	++++/++++	0,01/<0,01	0,02/<0,01
1 x 10^6 CFU/ml	++++/++++	0,02/++++	0,07/0,03	0,08/<0,01
1x 10^5 CFU/ml	++++/0,37	0,12/0,31	0,27/0,11	0,07/<0,01
1 x 10^4 CFU/ml	1,57/na	0,48/na	na/0,31	na/na

na Not analysed

Table 13. Recovery (%) of Pseudomonas sp. According to method no 3; swabbing

Minutes after contamination (min) 120 Dry / wet (d/w)	(d/w)	960 (d/w)	7200 (d/w)
1 x 10^8 CFU/ml	na/na	na/na	nd/0,02
1 x 10^7CFU/ml	na/na	0,19/0,11	nd/0,01
1 x 10^6 CFU/ml	na/3,37	0,35/0,45	nd/nd
1x 10^5 CFU/ml	0,08/0,86	0,10/0,11	nd/nd
1 x 10^4 CFU/ml	nd/0,33	0,10/0,17	nd/nd
1 x 10^3 CFU/ml	nd/nd	0,2/0,2	na/na
1 x 10^2 CFU/ml	0,01/nd	na/na	na/na

na Not analysed
nd Not detected

Table 14. Recovery (%) of Pseudomonas sp. according to method no 4; scraping

Minutes after contamination (min) wet (d/w)	120 (d/w)	960 (d/w)	7200 (d/w)
1 x 10^8 CFU/ml	na/na	0,24/0,03	nd/0,01
1 x 10^7CFU/ml	na/na	0,22/0,04	0,03/nd
1 x 10^6 CFU/ml	na/0,11	0,04/0,01	0,04/nd
1x 10^5 CFU/ml	0,03/0,04	nd/nd	0,05/nd
1 x 10^4 CFU/ml	0,03/0,01	0,18/0,12	na/na
1 x 10^3 CFU/ml	nd/nd	na/na	na/na
1 x 10^2 CFU/ml	nd/nd	na/na	na/na

nd Not detected
na Not analysed

Tables 10-14 shows the results from experiments with different number of microorganisms (CFU/ml) and longer time of contamination compared to previous experiments (table 3 – 8). The limit of growth / no growth seems to be related to the time of contamination and not the number of microorganisms in the contamination. There seem to be no difference in recovery between wet and dry samples. The results the first part of this study indicate more difference in the recovery between dry and wet samples compared to previously experiments (table no 3-8).

3.3 Scanning Electronic Microscopy (SEM)

SEM photos of the contaminated sample showed only growth on samples contaminated with high number of bacteria, suggesting that the bacteria penetrate into the wood and are trapped or killed there. Some scientists have suggested that some factors in the wood are bactericidal but it has never been confirmed so it is still just a suggestion. It has been shown that almost 75% of adherent bacteria on the wood surface were viable after 2 hours drying times (Abrishami et al, 1994). Another reason might be that when preparing the samples for SEM then some of the bacteria may be washed of during fixation. Scanning electron photomicrograph of samples is shown in appendix 8.

Evaluation of these surfaces with SEM shows wood samples to be roughest as expected. Samples of stainless steel and the plastic samples were though marked by grooves and crevices.

4 Conclusion

All measuring methods have advantages and disadvantages, especially related to standardising. The percentage recovery of microorganism is a function of the nature of the surface. It is possible that there is a fixed limit for removing the microbes from a wooden surface. A limit that is caused by the hygroscopic properties and porous structure of the wood. It is known that recovery from stainless steel is higher than wood, but the results from this study shows that different contamination time influences the recovery from all tested surfaces: wood, plastic and stainless steel. In our experiments, we observe a decline of recovery while the contamination time increases.

Although none of the measuring methods gives optimum results, we consider the contact and the swabbing method to be most convenient and suitable for the industry. The swab method is easy to perform, not destructive, quantitative and it is possible to use on all kinds of surfaces. The contact method is easy to perform and convenient for a screening of the hygienic conditions of the wood. If the number of microorganisms on a surface is low, it is possible to quantify the numbers of microorganisms by using the contact method. If the surface is very contaminated, the contact method will be qualitative. When testing for the red halophilic bacteria in the salt fish industry in Norway, it is sufficient to make a qualitative test, because it is required that no red halophilic bacterias to be present in the salted fish (Anon, 1997).

The SEM experiments support the knowledge about the porosity if the wood compared to plastic and stainless steel. The SEM studies can not help us choosing methods. The photos only shows that in the wood, bacteria can find lot of hiding places within the rough surface of wooden vessels. The photos show open porous cellular structure of wood.

5 Acknowledgements

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Appendix

App no 1: Halofile og osmofile mikrober (rødmidd og brunmidd). Bestemmelse i fullsaltede produkter

App no 2: Recipe for fish juice

App no 3: Testplans at FF

App no 4: Testplans at IFL

App no 5: Photos, measuring methods; 1 – 5

App no 6: Procedure for SEM, preparation of specimen

App no 7: Density of wooden samples

App no 8: SEM Photos, by Birna Guðbjörnsdóttir.

Halofile og osmofile mikrober (rødmidd og brunmidd). Bestemmelse i fullsaltede fiskeprodukter.

1. Formål og anvendelsesområde

Metoden kan anvendes for å påvise rødmidd i saltfisk. Metoden omfatter analyser av saltfisk som er produsert av torsk eller sei.
Rød- påvises etter at fortynninger av prøven er strøket ut på overflaten av en ferdigstøpt agarplate. Prøver for rødmidd inkuberes lyst ved 37 °C i 4-5 dager. Vekst av rødmidd kjennetegnes ved at de gir sterkt pigmenterte kolonier.

2. Rødmidd – medium (DSMZ – medium nr 97)

Gjærekstrakt	10 g
NaCl	250 g
Magnesiumsulfat, $MgSO_4$ x 7 H_2O	20 g
Mangansulfat, $MnSO_4$ X H_2O	0,2 mg
Jernsulfat, $FeSO_4$ x 7 H_2O	0,05 g
KCl	2,0 g
Na3 – citrat	3,0 g
Casaminosyrer	7,0 g
Agar (Oxoid L13)	20 g
Destillert vann	1000 ml

Løs opp stoffene under omrøring og juster pH til 7,4 og autoklaver ved 121 °C i 20 minutter.

3. Fortynningsmedium for rødmidd

NaCl	250 g
Pepton	1 g
Destillert vann	1000 ml

4. Prøveuttak

Foreta prøveuttak etter vanlige bakteriologiske prinsipper, og slik at prøven danner et mest mulig gjennomsnitt av fisken som skal kontrolleres.

5. Fremgangsmåte

Forbehandling
Utfør forbehandling og fortynning av prøvene i samsvar med NMKL-metode nr. 91

6. Utsæd, inkubasjon og avlesing

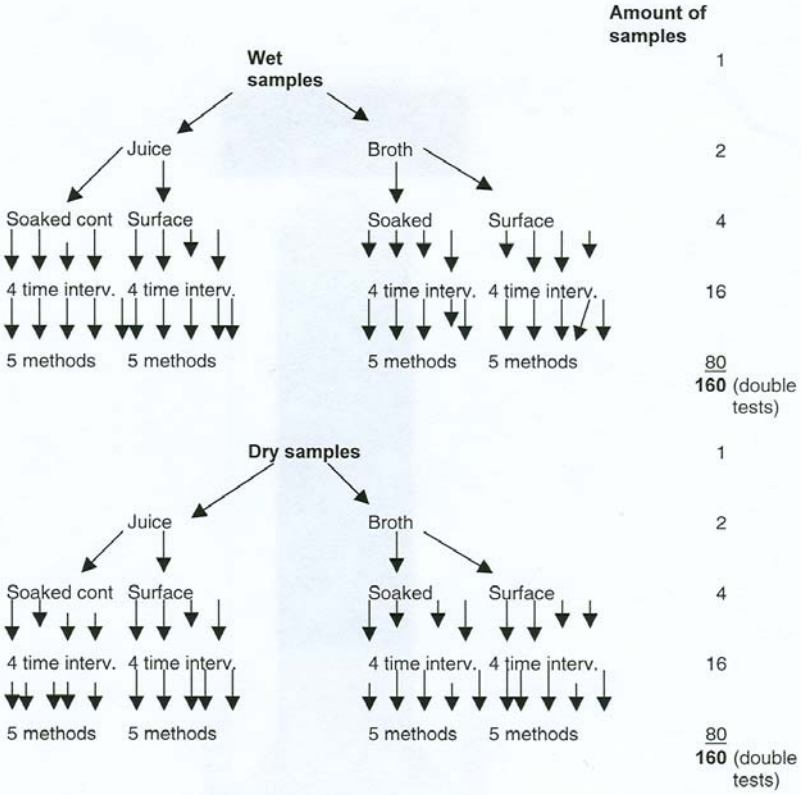
Overfør 0,1 ml fra passende fortynninger av prøvematerialet til en Petrisskål med ferdigstøpt rødmidd medium. Stryk prøvematerialet inn i mediet med en steril og avkjølt glasstav. Petrisskålene pakkes inn i plastikkposer og inkuberes lyst ved 37 °C. Les av skålene etter 4-5 dager inkubering. Vekst indikeres ved utvikling av røde kolonier.

Appendix 2
Preparation of fish juice

1. 1 part fish: 2 parts of distilled water or 1 part fish: 1 part of distilled water.
2. Boiled for 2 minutes.
3. Filtered with doubled coffee-filter.
4. Supplemented with 3 % NaCl (or other chemicals) or the consentration we wish to consider and pH should be relevant.
5. Autoclaved at 121 °C for 15 minutes.

Appendix 3
Testplan FF

Experiment no 1



Appendix 3
Testplan FF

Experiment no 2

Test plan for further experiments at Fiskeriforskning

In order to improve the detection level of the methods (no 3 and no 4) we will do some more experiments.

Variables: Incubation time of *Halobacterium salinarum* on the wooden samples; 2 hrs, 16 hrs, 5 days, 12 days
Methods; scraping and swabbing
Time of incubation in liquid media; 5 hrs, 16 hrs

Contamination; *Halobacterium salinarum* in salt broth
Concentration of microorganisms in the contamination; three levels, approx 10^6, 10^4 and 10^2 CFU/ml

We will use only dry samples

Control: Dry samples "contaminated" with pure liquid media; salt broth

0,5 ml of contamination added on the wooden surface
⇓
Incubation for 2 hrs, 16 hrs, 5 days, 12 days at 37 °C
⇓
Sampling; swabbing and scraping
⇓
Incubation of swab / treflis (approximately 0,25 g) in a test tube containing 3 ml liquid growth media (DSMZ no 97, without agar) in 37 °C in 5 and 16 hrs. Constant stirring.
⇓
Sampling; 0,1 ml of sample on the agarplate. No dilution required, because we will study if it will grow or not. Incubation at 37 °C.

Parallel experiments;

1. Study microbial growth in the wooden sample
We will contaminate some wooden samples with "heavy loaded" *Halobacterium salinarum* "soup", incubate the samples under optimum conditions, and then brake the sample into two pieces. Hopefully we will be able to detect the growth and distribution of the microorganisms in the wooden sample.

2. Standardisation of the scraping
We will try to do some experiments with an "høvel" in order to standarisize the sampling instead of using the scalpel.

Appendix 4
Testplan IFL

Experiment no 1. Measuring methods.

				No samples
		Wet samples		1
	Juice		Broth	2
	4 time interv		4 time interv	8
	5 methods		5 methods	40
				80 double test
		Dry samples		1
	Juice		Broth	2
	4 time interv		4 time interv	8
	5 methods		5 methods	40
				80 double test
		Plast samples		1
	Juice		Broth	2
	4 time interv		4 time interv	8
	3 methods		3 methods	24
				48 double test
		Stainless steel		
		Juice		1
		4 time interv		4
		1 method		4
				8 double test

Appendix 4
Testplan IFL

Experiment no 2. Different levels of contamination

In order to test the detection level of the methods (no1,3 and 4) wood samples were contaminated by different level of micro-organisms.

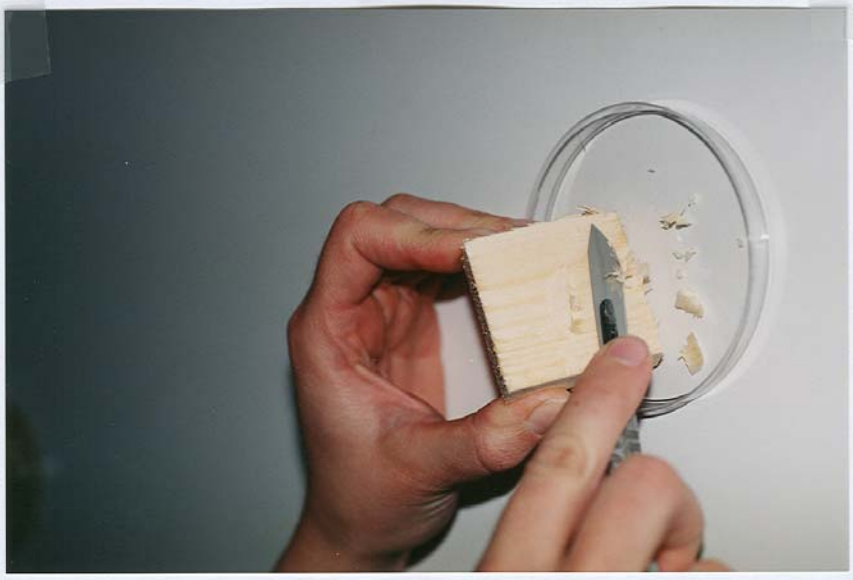
- *Pseudomonas* sp incubated in fishjuice used as contamination
- Contamination time: 2hrs, 16 hrs and 5 days
- Methods: 1, 3 and 4
- Concentration of microrganisms in the contamination: 10^3-10^9 CFU/ml

Appendix 5
Measuring method no 1; contact method
Photo: Grete Lorentzen

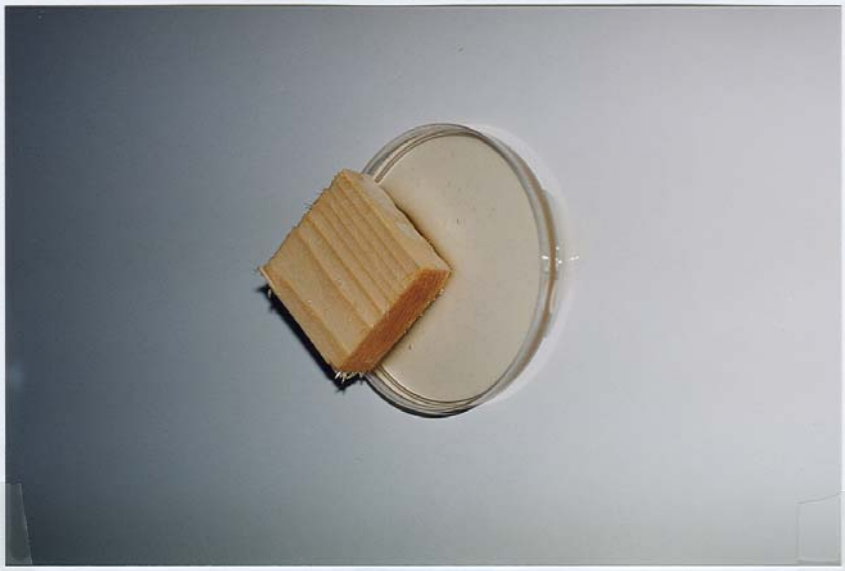
Appendix 5
Measuring method no 2; soaking of sample in water
Photo: Grete Lorentzen

Appendix 5
Measuring method no 3; swabbing
Photo: Grete Lorentzen

Appendix 5
Measuring method no 4; scraping
Photo: Grete Lorentzen



Appendix 5
Measuring method no 5; liquid growth medium on the surface
Photo: Grete Lorentzen



Appendix 6

Scanning Electronic Microscopy (SEM). Preparation of the specimen.

1. Each wood section is placed in a vial containing 2 % gluteraldehyde in 0.025 mol/l phosphate buffer pH 7.0 for at 16 -24 hours at 4°C.

2. The section washed twice for 20 min with 1 ml aliquots of phosphatebuffer

3. Dehydrated in an ascending graded ethanol series

10% v/v ethanol for 15 min
30% v/v ethanol for 15 min
50% v/v ethanol for 15 min
70% v/v ethanol for 15 min
90% v/v ethanol for 15 min
100% v/v ethanol for 15 min

4. Drying

The dehydrated samples are transferred to the critical point drying apparatus and infiltrated with liquid carbon dioxide under pressure for 3 hours. The temperature is then raised until the critical point for carbon dioxide is reached and the density of its vapour is the same as that of its liquid phase. Venting off the vapour at this stage result in a dry sample free from any surface tension artefacts.

5.
Finally the specimen is cold coated for 3 minutes (sputter coating with gold in argon) before examination with SEM

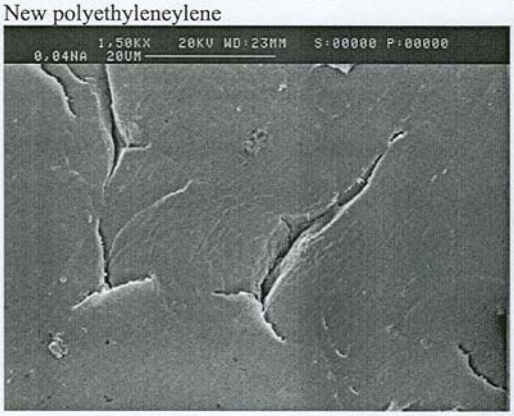
Appendix 7
Densities of wooden samples

Nr.	Length	Width	Thick-ness	Wet weight	Dry weight	Moisture	Density at actual moisture content= mu/vu	m0/vu	Nr.	x	
A35	46,70	47,30	20,93	18,28	16,26	12,4	395	352	1	1	395
A38	46,40	48,30	21,08	21,26	18,89	12,5	450	400	2	1	450
A73	46,60	47,20	20,88	18,07	16,01	12,9	393	349	3	2	393
A74	46,40	48,80	20,87	19,33	17,12	12,9	409	362	4	2	409
A110	46,50	48,20	20,90	20,40	18,11	12,6	435	387	5	3	435
A111	46,75	47,00	20,88	20,07	17,83	12,6	437	389	6	3	437
A144	46,10	47,80	20,39	19,56	17,33	12,9	435	386	7	4	435
A149	46,70	47,20	20,78	21,28	18,93	12,4	465	413	8	4	465
A162	46,50	46,20	20,45	17,52	15,56	12,6	399	354	9	5	399
B32	46,60	47,10	20,25	21,98	19,46	12,9	495	438	10	1	495
B37	46,70	48,50	20,57	22,50	19,83	13,5	483	426	11	1	483
B66	43,10	47,20	20,12	20,36	17,93	13,6	497	438	12	2	497
B75	43,10	48,40	20,46	20,42	17,96	13,7	478	421	13	2	478
B100	46,50	47,20	19,75	19,97	17,62	13,3	461	406	14	3	461
B113	46,70	48,10	20,18	22,03	19,42	13,4	486	428	15	3	486
B134	43,00	47,00	19,71	18,61	16,55	12,4	467	415	16	4	467
B147	44,90	48,20	20,19	21,23	18,74	13,3	486	429	17	4	486
C35	46,20	49,20	20,28	21,02	18,53	13,4	456	402	19	1	456
C36	46,60	48,00	20,51	20,03	17,59	13,9	437	383	20	1	437
C69	46,60	47,90	19,75	19,37	16,99	14,0	439	385	21	2	439
C72	46,40	47,80	19,72	19,96	17,56	13,7	456	401	22	2	456
C107	46,50	47,10	19,75	19,78	17,36	13,9	457	401	23	3	457
C110	46,70	48,60	19,57	19,80	17,31	14,4	446	390	24	3	446
C135	46,50	47,00	19,80	19,13	16,92	13,1	442	391	25	4	442
C140	45,70	48,50	19,68	18,85	16,56	13,8	432	380	26	4	432
C171	46,40	46,90	19,51	19,36	17,06	13,5	456	402	18	5	456
D36	46,60	47,20	19,68	19,28	16,95	13,7	445	392	27	1	445
D39	46,70	47,50	19,87	19,49	17,09	14,0	442	388	28	1	442
D74	46,70	47,80	20,02	19,59	17,16	14,2	438	384	29	2	438
D75	46,60	47,80	19,66	19,25	16,87	14,1	440	385	30	2	440
D113	46,70	47,90	19,44	19,77	17,28	14,4	455	397	31	3	455
D114	46,50	47,50	19,78	19,29	16,83	14,6	442	385	32	3	442
D152	46,60	47,50	19,43	20,44	17,88	14,3	475	416	33	4	475
D153	46,70	47,50	19,64	20,15	17,66	14,1	463	405	34	4	463
E33	46,70	48,90	20,67	23,21	20,47	13,4	492	434	35	1	492
E36	46,50	47,00	20,64	21,64	19,00	13,9	480	421	36	1	480
E71	46,60	48,20	20,78	21,78	19,12	13,9	467	410	37	2	467
E74	46,60	47,80	20,65	22,12	19,38	14,1	481	421	38	2	481
E107	46,70	48,60	21,00	23,24	20,31	14,4	488	426	39	3	488
E112	46,60	47,30	20,80	22,23	19,44	14,4	485	424	40	3	485
E147	46,70	48,40	20,54	22,94	20,05	14,4	494	432	41	4	494

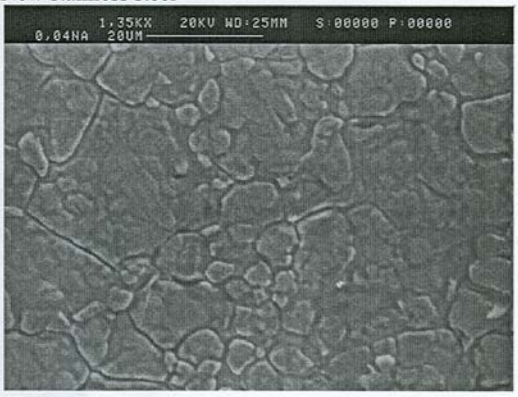
F35	46,50	47,80	21,10	21,24	18,65	13,9	453	398	42	1	453
F40	46,70	49,20	20,97	22,61	19,77	14,4	469	410	43	1	469
F73	46,70	48,40	21,32	22,86	20,00	14,3	474	415	44	2	474
F80	46,30	48,80	21,21	20,28	17,69	14,6	423	369	45	2	423
F118	46,60	48,50	21,04	20,51	17,90	14,6	431	376	46	3	431
F145	46,60	48,20	20,68	22,76	19,87	14,5	490	428	47	4	490
F146	46,50	48,30	20,76	22,57	19,73	14,4	484	423	48	4	484
G37	43,20	47,80	19,97	18,26	16,11	13,3	443	391	49	1	443
G40	46,50	46,80	20,37	19,58	17,17	14,0	442	387	50	1	442
G71	43,60	48,00	19,90	18,64	16,38	13,8	448	393	51	2	448
G78	46,50	46,50	20,11	19,48	17,09	14,0	448	393	52	2	448
G109	46,70	48,10	19,95	20,00	17,57	13,8	446	392	53	3	446
G116	46,40	46,40	19,96	19,08	16,75	13,9	444	390	54	3	444
G149	46,70	48,20	19,87	19,72	17,35	13,7	441	388	55	4	441
G154	45,00	46,00	19,95	18,57	16,37	13,4	450	396	56	4	450
H38	46,40	48,40	19,88	20,51	18,13	13,1	459	406	57	1	459
H39	46,60	46,60	19,96	19,90	17,55	13,4	459	405	58	1	459
H74	46,50	48,50	19,74	20,24	17,82	13,6	455	400	59	2	455
H75	46,10	46,40	19,87	18,88	16,60	13,7	444	391	60	2	444
H106	46,70	48,00	20,11	20,36	17,92	13,6	452	398	61	3	452
H113	46,30	46,80	20,00	19,33	16,98	13,8	446	392	62	3	446
H144	46,60	48,50	20,53	21,14	18,63	13,5	456	402	63	4	456
H147	46,50	46,80	20,25	20,37	17,95	13,5	462	407	64	4	462

Scanning electron photomicrograph of new food cantact surface: (polyethylene, stainless steel and wood)

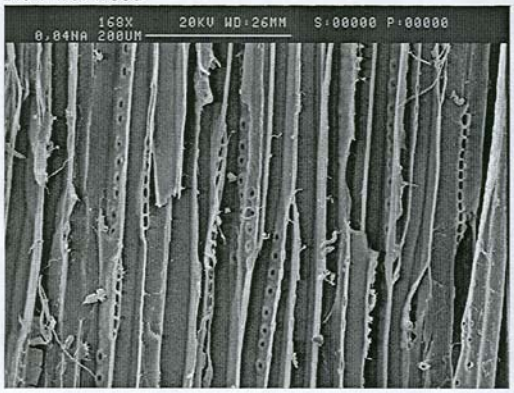
New polyethyleneylene



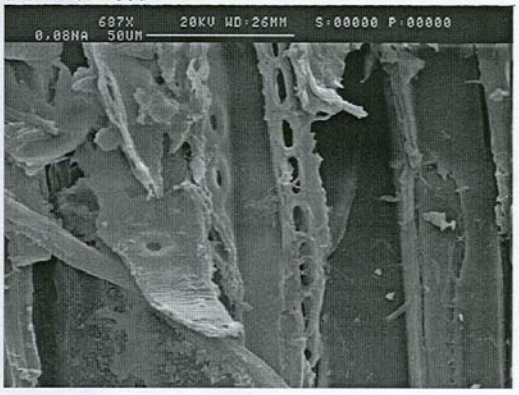
New stainless steel



New wet wood



New wet wood

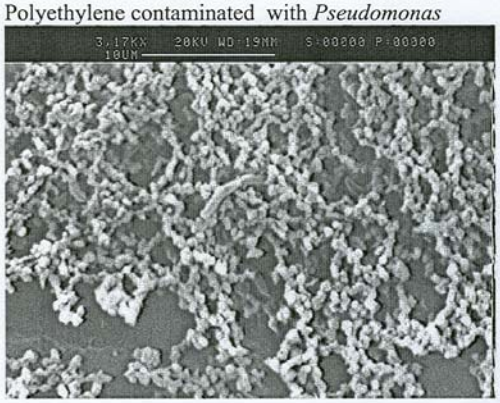


New dry wood

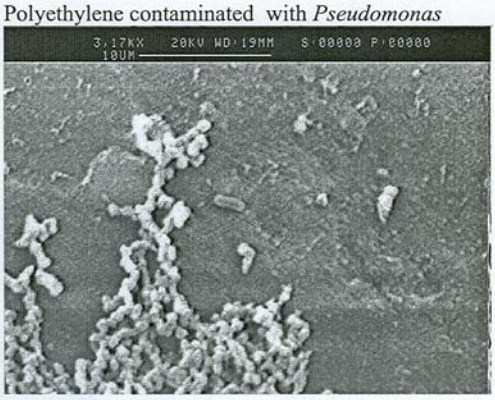
New dry wood

Scanning electron photomicrograph o*f Pseudomonas* attached to polyethylene, stainless steel and wood

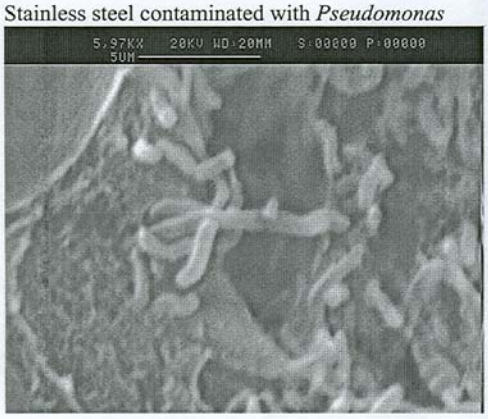
Polyethylene contaminated with *Pseudomonas*



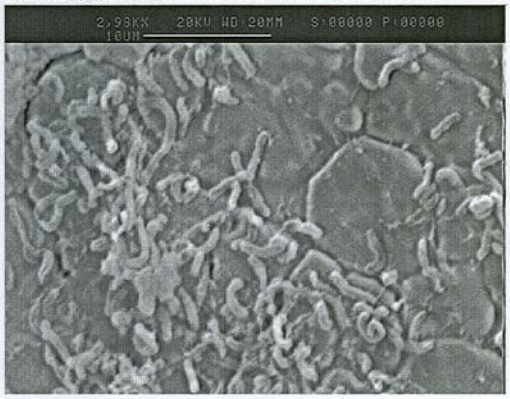
Polyethylene contaminated with *Pseudomonas*



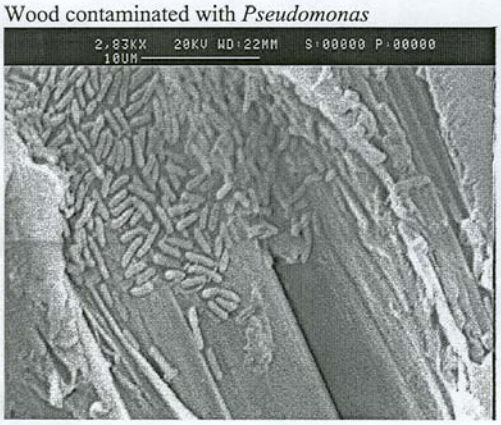
Stainless steel contaminated with *Pseudomonas*



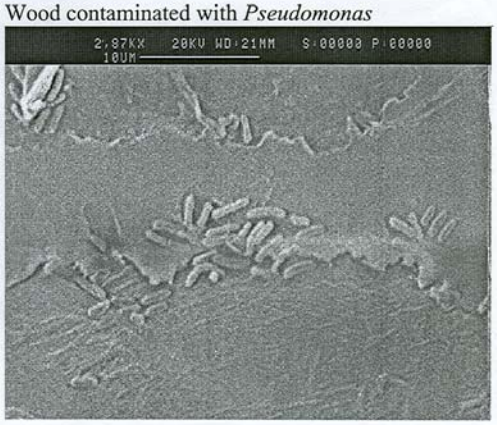
Stainless steel contaminated with *Pseudomonas*



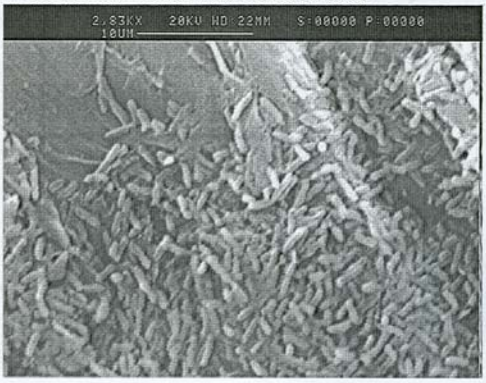
Wood contaminated with *Pseudomonas*



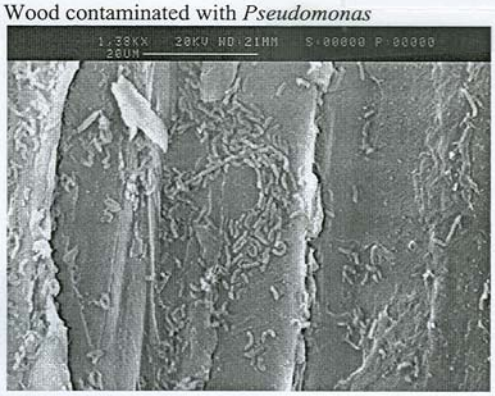
Wood contaminated with *Pseudomonas*



Wood contaminated with *Pseudomonas*

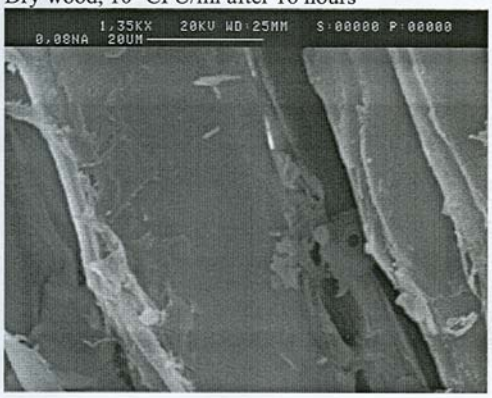


Wood contaminated with *Pseudomonas*

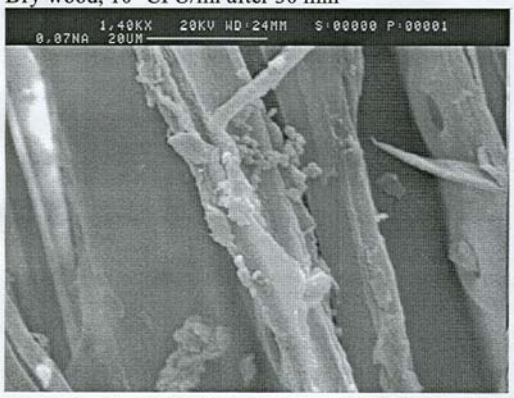


Dry wood, 10^5 CFU/ml after 30 min

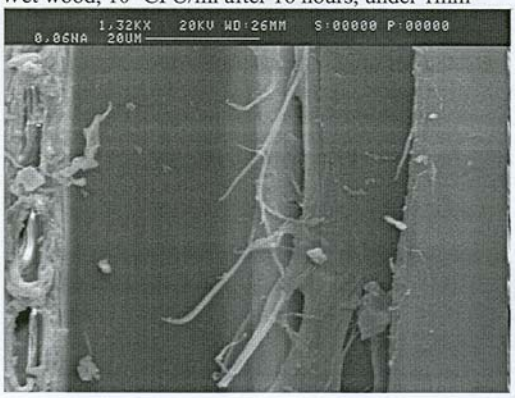
Dry wood, 10^6 CFU/ml after 16 hours



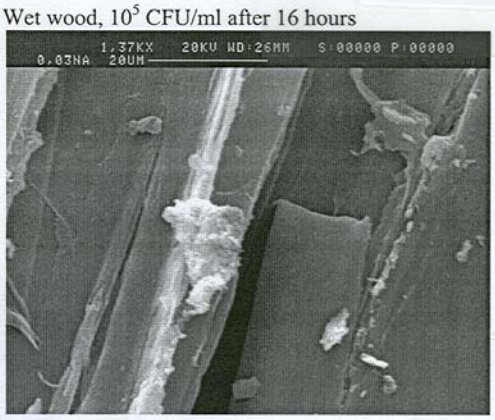
Dry wood, 10^6 CFU/ml after 30 min



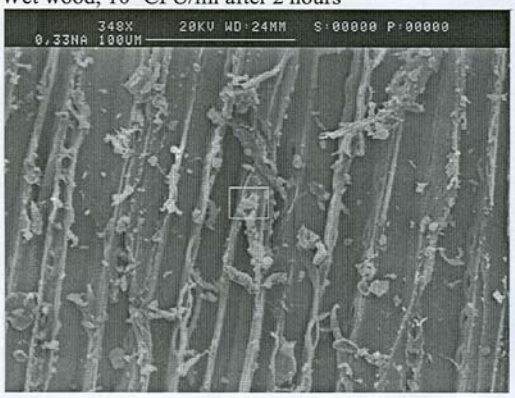
Wet wood, 10^7 CFU/ml after 16 hours, under 1mm



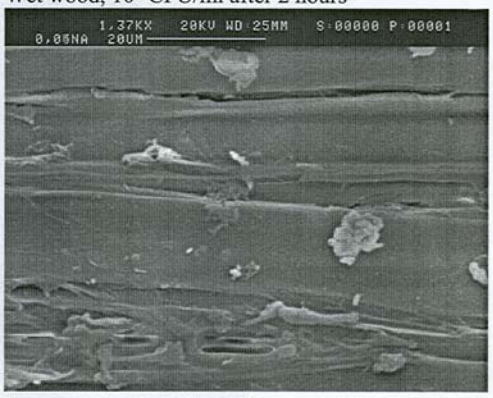
Wet wood, 10^5 CFU/ml after 16 hours



Wet wood, 10^5 CFU/ml after 2 hours

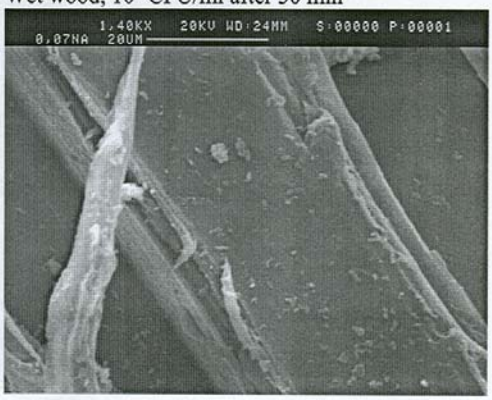


Wet wood, 10^6 CFU/ml after 2 hours



We wood, 10^4 CFU/ml after 30 min

Wet wood, 10^6 CFU/ml after 30 min



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