Report

How to Clean with Less Water

Catalogue of ideas

Anette Granly Koch, Karen Sørensen, and Emma Bildsted Petersen

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Introduction

Aim

The aim of this report is to summarize ideas for how to reduce the water consumption during cleaning in the meat industry.

It is not a complete list of ideas/possibilities, but a list of technologies that were investigated in tests or by literature survey during the project.

Cleaning in the meat industry

Traditional cleaning procedure

The traditional cleaning procedure in the meat industry involves large volumes of water and liquid chemicals. The current cleaning programme can be divided into the following subroutines: 1) Prerinsing with lukewarm water to remove solid waste, 2) Application of cleaning detergent, 3) Rinsing, 4) Disinfection, 5) Rinsing, 6) Drying.

The food production areas in the meat industry are mainly open surfaces (belts and steel surfaces), which is why most of the cleaning is performed manually using a low pressure system. Lukewarm water (50-55°C) is used for removal of all loosely bound soil before chemical agents are applied. Alkaline and acidic products are rotated during the week for optimal cleaning and disinfection effect. Alkaline (with chlorine) and acidic products are applied for removal of protein and limescale depots, respectively (Skaarup, 1985).

Manual removal of solid waste – pre-cleaning

Manual scraping

Principle

The production area must be cleared from raw materials, products, packaging material etc. before being handed over to the cleaning company. This includes removal of meat from conveyor belts and floors using hands or scrapers. Water should not be used for pre-cleaning.

It is a general problem in the meat industry that the clearance of production areas is insufficient. The responsibility for clearance of the individual areas should be assigned to specific people, and it should be well-defined, to which extent areas must be cleaned.

Effect

The direct effect of increased focus on clearance has not been tested within this project.

The water consumption for cleaning will, however, depend on how thoroughly the production area has been cleared, as water is otherwise used to rinse away product residues from equipment, surfaces, and floors. The larger the residues, the more water is needed to move the products around.

Product residues representing a value can be used for feed production, and if large quantities of meat end up in the sewers, it will result in clogging and costs for sludge suction. Slaughterhouses also pay a volumetric contribution for their wastewater and the amount of organic matter in it.

Areas for use

Pre-cleaning should be performed in the entire production area.

Assessment (advantages and disadvantages)

Advantages of manual scraping:
- Less water consumption
- Better use of waste material
- Less frequent clogging of sewers
- Lower amount of wastewater
- Less organic matter in the wastewater

Disadvantages of manual scraping:
- Increased instruction of operators

Wet vacuum cleaning

Principle

Wet vacuum cleaning is similar to regular household vacuum cleaning, and it can be used as a tool for better clearance of floors before cleaning with water, both during and at the end of the production day.

The vacuum system is comprised of a tube connected to a hose, which is then connected to a trolly. A mouthpiece with wheels is attached to the end of the tube for ergonomic purposes. Waste is collected in the trolly to be emptied when full.

Effect

Danish Technological Institute (DTI) (Mortensen & Pontoppidan, 1991) has tested the use of a wet vacuum cleaner in a slaughterhouse and analysed aspects of water consumption, time, collected waste, and water pollution in comparison to traditional cleaning procedures. 1,200 pigs were slaughtered during the test period.

- Water consumption: Reduction from 4.6 m^3 to 0.4 m^3 (91.3%)
- Time: Reduction from 1.74 h to 1.56 h (10%)
- Collected waste: Increased from 0.2 kg to 0.8 kg (400%)
- Water pollution: Overall reduction of 140 mg BI_5/l

Areas for use

The test was performed in the 'clean production area', which is where the cutting and dividing parts produce the most floor waste. It has been suggested that wet vacuum cleaning procedures could also be applied to the bleeding area. The collected waste should comprise material suited for a given purpose as there is no subsequent sorting.

Assessment (advantages and disadvantages)

Advantages of wet vacuum cleaning:
- Less water consumption
- Less water pollution
- Less cleaning time
- Economic rentable
- Better use of waste material

Disadvantages of wet vacuum cleaning:
- Increased instruction of operators
- Switch between different equipment
- Large residues cannot be collected with the system (blockage)
- Mostly used to collect soft material and thereby risk of blockage

- Limited capacity of the trolly compartment

Ice pigging

Principle

Ice pigging can be used for cleaning tubes. The ice slurry, comprised of small crystals, is pushed through the pipes under pressure to carry out the cleaning process. The slurry is pumped into a pipe like a liquid but moves through the pipes like a solid plug, detaching contaminations as well as soil and carrying it out of the tube.

Ice pigging is used in the drinking water and wastewater industry and is used instead of rinsing with water.

The technology cannot be used on open surfaces as the cleaning effect of the slurry comes from the applied pressure.

Effect

The manufacturer (Suez) of ice pigging illustrates the effect on their homepage (Figure 1) where they make a visual comparison of cleaned tubes with water (hot and cold) and ice pigging.

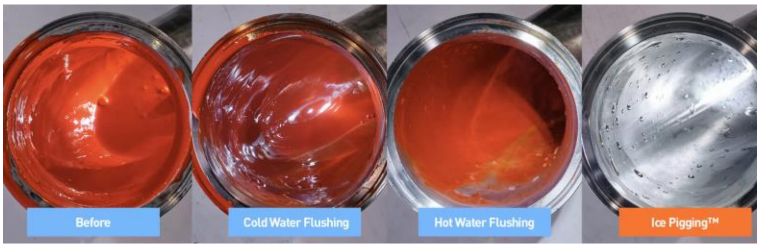

Figure 1. The cleaning effect of ice pigging compared to the use of hot or cold water.

Areas for use

Pipe systems.

Assessment (advantages and disadvantages)

Advantages:
- Water and time saving
- Higher cleaning effect
- Can remove the last part of products in pipes, which might be used for food or feed instead of being discarded

Disadvantages:
- Cannot be used on open surfaces

Automatic cleaning systems

Nozzle bars for belt cleaning

Principle

On a daily basis, large amounts of water and time are used for cleaning belts in the production areas. The cleaning is often performed manually by a cleaning operator. Measurements performed by DTI have shown that there is large variation in how much water/time the induvial operator uses for cleaning belts. The water consumption varies from 25 to 141 l per meter belt, and it has been observed that many operators pre-rinse the belt for a longer time than needed. Furthermore, it has been observed that most operators have too long a distance from the cleaning nozzle to the surface, and that they move the water hose too much during cleaning. This decreases the mechanical force making the cleaning less effective (Sørensen & Bildsted Petersen, 2023).

It is possible that the use of automatic cleaning, such as nozzles bars on belts, would make the cleaning more consistent and effective, as the nozzles are installed on the belt with the optimal distance/angle. Furthermore, it could give the cleaning operators more time for floor scraping resulting in less organic matter ending up in the sewers.

System Cleaners is an example of a supplier who can provide several solutions for belt cleaning (System Cleaners, Automated cleaning solutions).

Effect

The direct effect of automatic cleaning was not tested and measured. The recommendation is based on observations of the current process.

Areas for use

Alle types of belts.

Assessment (advantages and disadvantages)

Expected advantages:
- More consistent cleaning
- Water and time savings

Expected disadvantages:
- Costs associated with installation and maintenance

Steam vacuum system for belt cleaning

Principle

Steam vacuum cleaning can be used for cleaning various belts or other types of surfaces during production as well as after the daily production. The steam loosens adherent soil, product debris or bacteria, which is then sucked up by the vacuum. The vacuum also removes vapour/water leaving the surface dry.

Some devices are designed as mobile units for automatic cleaning of conveyor belts including both flat and modular belts. Other devices are built as a portable steam vacuum generator with a silicone head containing nozzles (tubes). Steam is applied though channels in the nozzle that are in direct contact with the surface (e.g., Steam Vac).

Effect

DTI has tested and compared the effect of steam/vacuum cleaning of flat conveyor belts with conventional belt scraping (Bøegh-Petersen, 2015). The two techniques were tested by simulating a production scenario in the meat industry where the belt was continuously contaminated with a thin layer of meat juice/emulsion. After approx. 50 minutes of continuously contamination and steam cleaning, the aerobic plate count at 20ºC was reduced from 4.5-5.0 log cfu/cm^2 to < 1 cfu/cm^2. The use of a belt scraper led to a 1-2 log reduction after 50 min.

Rasmussen, 2016 (cf. Storm Høgsbro, 2016) showed that at flat smooth conveyer belts, the number of bacteria could be reduced by 3-4 log cfu/cm^2. On cutting plates with an initial count of 850 cfu/cm^2, the number of bacteria was reduced to below 10 cfu/cm^2.

DTI also tested the steam vacuum system as a tool for intermediary cleaning of conveyer belt (interlock/modular belt) during prolonged production. The maximum reduction obtained was 1.3 log cfu/cm^2 after nine rounds of steam/vacuum treatment. The reason for this lower effect compared to a flat/smooth conveyor belt might be cutting damage and several spaces between the lamellas. During the study, it was also observed that the longer treatment and production time the less effect. Meat was denatured and became stocked to the surfaces of the conveyor belt (Storm Høgsbro, 2016).

Areas for use

Steam vacuum cleaning could be used for:
- Cleaning and disinfection of conveyor belts in low soil areas
- Cleaning of conveyor belts in areas with a high load of soil if the surfaces are initially scaped

Assessment (advantages and disadvantages)

Advantages:
- Water savings
- Can be used during production and for end-of-the-day cleaning

Disadvantages:
- A high steam temperature can lead to discoloration of the belt
- Solutions for cleaning of belts with a high level of soil are missing. E.g., a combination of a belt scraper and stream vacuum cleaning
- Continuous use for a long period of time can result in burnt residues on the surfaces
- Cannot clean spaces between lamella in conveyer belts, e.g., interlock belt

Optimized cleaning programme

Use chemicals before pre-rinsing

Principle

The most time-consuming step in the cleaning procedure and where large volumes of water are used is during the pre-rinsing when meat residues are removed from surfaces. It is suggested that the water consumption can be reduced if a chlorinated cleaning product is used before the pre-rinsing step, as soil would easier be dissolved and removed from the surfaces. It would, however, most likely be necessary to apply a cleaning detergent for a second cleaning, as the soil level in most areas in the meat industry is too high to remove it all at once (the cleaning detergent will not penetrate to the lowest layer of soil). This cleaning programme will increase the number of steps for cleaning; *1) Chlorinated cleaning detergent, 2) Rinsing with water, 3) Chlorinated cleaning detergent, 4) Rinsing, 5) Disinfection, 6) Rinsing, 7) Drying.*

One of the major limitations when using this cleaning programme is that the consumption of chemicals will increase, which does not meet with the sustainability agenda. Furthermore, it is possible that the time saved by using a cleaning detergent as the first step is spend on the prolonged contact period for the chemicals and shifting between different nozzles and products.

Effect

The effect has not been tested.

Areas for use

Using 2-times cleaning detergent is most relevant in areas with soil that are difficult to remove.

Assessment (advantages and disadvantages)

No comments.

Cleaning with higher pressure

Principle

The current cleaning procedure uses low water pressure with 25 bars. It is hypothesized that using a medium water pressure will lead to both time and water savings, as a larger volume of water will reach the surfaces.

Effects

System Cleaners has compared low pressure cleaning using 25 bars with high pressure cleaning at 40 bars. The results of their comparison showed that approx. 84% more water reached the surface when using low pressure, which speeds the removal of soil and lowers the water consumption (System Cleaners, Low pressure cleaning with boosted water). Moreover, the aerosol formation increased with high pressure cleaning. Aerosols can transfer contaminated particles to already cleaned surfaces and cause respiratory problems for the cleaning staff.

Based on these results, it cannot be recommended to use high pressure cleaning. However, it has not been tested if cleaning with medium pressure (using e.g., 30-35 bars) will have the same limitations. It would, however, be difficult for many food productions to deliver a higher pressure than what is used today. Further tests are thereby opted out.

Areas for use

No comments.

Assessment (advantages and disadvantages)

No comments.

Water-saving nozzles

Principle

The water consumption for daily cleaning in the meat industry is high, and it has previously been estimated that 30 to more than 50% of the 'cleaning water' is used for pre-cleaning of equipment and surfaces. For pre-cleaning, nozzles with a capacity of 40-50 l/min is often used, and the water temperature is 50-55°C. The same nozzle is further used for chemical rinsing.

Preliminary tests performed by ISS have shown that the water consumption used for pre-rinsing can be reduced by using a nozzle with a lower capacity of 30 l/min without affecting the efficiency, cleaning quality, or the working environment.

Effect

Pre-rinsing using a nozzle with a capacity of 30 l/min was tested in a cutting and deboning area at a selected slaughterhouse (Sørensen & Bildsted, 2023a). The nozzles were tested during four production weeks, during which a baseline of the current process and water consumption was established for the first two test weeks, and the new nozzle was tested for the remaining period.
The water consumption was reduced by 10% when the pre-rinsing was performed with a 30 l/min nozzle instead of a 40 l/min nozzle. The same amount of time was used for the cleaning, and the same level of cleaning was achieved.

The saving potential is assessed as being higher, as the nozzle was not used consistently by all the cleaning staff during the test period (Sørensen & Bildsted, 2023a,b).

Areas for use

It is not known if a water saving nozzle is optimal for all departments. It is recommended that the use of these nozzles is gradually introduced to the companies' various departments, as the implementation requires increased focus from the supervisor.

Assessment (advantages and disadvantages)

Advantages:
- Water savings

Disadvantages:
- Requires training of the cleaning staff.

Water temperature

Principle

The water temperature is important for pre-rinsing, as the cleaning effect is highly dependent on it. Protein and fats are the most common types of soil in the meat industry. If the water is too hot (≥ 60-80°C), proteins will denature and precipitate on surfaces making them difficult to clean. On the other hand, if the water temperature is too low, fats will not be effectively removed, as they are not melted. Usually, the temperature is set to approx. 5°C above the melting point, corresponding to a water temperature of more than 50°C for pig fat. Lard from pigs has a melting point between 32°C and 45°C, therefore a combination of cleaning methods at 42°C might remove fats efficiently (Skaarup, 1985).

Based on these criteria for proteins and fats, a water temperature of 45-55°C would be optimal for cleaning in a pig slaughterhouse or a production site where meat is processed. When the water temperature is adjusted, it is important to account for the decrease in temperature from the outlet of the water (in this context: the nozzle) and the surface that the water reaches.

Effects

Not relevant.

Areas for use

Not relevant.

Assessment

Not relevant.

(advantages and disadvantages)

Chemical substitution

Combined cleaning and disinfection products

Principle

Cleaning and disinfection can be combined into one operation using a product containing both cleaning detergents and a disinfectant (C&D product). In some cases, these products can replace the traditional 2-step procedure in which surfaces initially are cleaned with an alkaline detergent (containing chlorine) followed by disinfection with sodium hypochlorite (NaOCl) after an intermediate rinsing with water.

The use of C&D products therefore offers both time and water savings. There is a broad range of products available, which include chloralkaline foam. The average contact time for C&D products is 5-20 min.

Effect

The use of combined C&D products has been tested by DTI (Bildsted & Koch, 2023). The water consumption can be reduced by approx. 1/3 if a combined C&D product is used instead of the conventional 2-step procedure.

Combined C&D products can, however, not be used in high soil areas of a food production, as the presence of cleaning detergents and organic residues limits the disinfection effect.

The long-term effect of combined C&D products in a low soil area has been tested in a pilot plant setup. For 3.5 weeks, a conveyor belt (modular) and steel surfaces were daily soiled with meat inoculated with bacteria and after 18 hours' simulated production, the surfaces were cleaned using a combined C&D product. An acceptable disinfection effect was obtained on the steel surface, while the effect on the conveyor belt was very limited although the belt was visually clean.

Areas for use

Based on these test results, is it not recommended to replace the daily 2-step procedure with the use of a C&D product in high soil areas or in areas with modular conveyor belts.

Assessment (advantages and disadvantages)

Advantages:

- Time and water savings

Disadvantages:

- Low disinfection effect on belts and in high soil areas
- Can only be used in low soil areas

No-rinse disinfectant

Electrolyzed water

Principle

Electrolyzed water (EW) is a chlorine-based disinfectant, which is approved for use on food-contact surfaces without rinsing with water. The disinfectant is made by electrolysis of saltwater using a generator, in which hypochlorous acid (HOCl) is produced.

EW is considered a "green" alternative compared to sodium hypochlorite (NaOCl), as the same disinfection effect can be obtained with a lower concentration of chlorine. The concentration of free chlorine in EW and NaOCl when used in the food industry is approx. 200-500 ppm and 1,000 ppm, respectively. EW can be generated in three forms: alkaline (pH 9-13), acidic (pH 5-2), and as a neutral solution (pH 7-8).

EW can be made on-site when needed or purchased in a ready-to-use format. The shelf stability of EW is limited as the active component is degraded in its original components over time. The degradation is accelerated when the solution is exposed to light and oxygen.

Effect

DTI has evaluated and compared the effect of EW water (pH 7-8, 200 ppm) with NaOCl during a series of tests in pilot plant (Bildsted, 2020). The disinfectant was produced using a generator, and the solution was used shortly after manufacturing (stored in a closed container).

Surfaces (30 x 20 cm) of stainless steel were contaminated with a cooked meat emulation containing a cocktail of bacterial spoilers or *Listeria monocytogenes*. The surfaces were cleaned and disinfected using the traditional 2-step procedure with an alkaline cleaning detergent and disinfection either with sodium hypochlorite followed by rinsing with water or disinfection with EW without rinsing.

The disinfection effect of the two products was evaluated by analysing the microbial load after the individual cleaning steps and by visual control. The contact time of sodium hypochlorite was 10 min (as recommended by the manufacturer). The microbial level after disinfecting with EW was analysed after 30 min of contact.

The disinfection effect of EW varied from day-to-day despite using the same cleaning procedure. The results from the test day showed that the disinfection effect of EW was as high as when NaOCl was used. The following week, the test was repeated, and results from this test showed that the disinfection effect of EW was significantly lower than NaOCl.

It is stated by many manufacturers that EW is less corrosive against e.g., stainless steel, as the concentration of chlorine is lower, and the pH of the solution can be produced with a neutral pH.

DTI has performed corrosion tests with two different EW products:

1. ECA water (ATC-Global, pH 7-8, 200 ppm chlorine)
2. Neuthox (Danish Clean Water, pH 4.9, 500 ppm chlorine)

Neuthox can replace the use of hot water cabinets for decontamination of knife blades on the slaughter line.

No sign of corrosion was seen on stainless steel surfaces when ECA water was applied to the surfaces for two months. The surfaces were washed with water and a brush every day before EW was applied in order to simulate the normal cleaning cycles in the meat industry. The surfaces were stored at 9-10°C during the tests, Bildsted (2020).

When Neuthox was tested, signs of corrosion were seen after 1-2 weeks on cans, knifes, and capsules, and after 8 weeks when used on stainless steel surfaces. The surfaces/objects were stored at 9-10°C during the tests (Koch, 2022).

Neuthox has also been tested as an alternative to hot water disinfection of knifes. Neuthox had the same microbial effect as 82°C hot water disinfection of knifes. However, coating of the surfaces was observed (Rasmussen & Christensen, 2014a).

Areas for use

EW can be used as a disinfectant in all areas of a production. It is, however, important to bear in mind that variable disinfection effects were measured, and that some EW products caused corrosion if they were used without rinsing, depending on the pH of the solution.

Assessment (advantages and disadvantages)

Advantages:

- Less use of chlorine
- No rinse (not applicable on metal due to rust formation)

Disadvantages:

- Variable effect, maybe because of variable production in the generator
- Corrosion

Inspexx

Principle

Inspexx (Ecolab) is an acid-based disinfection agent approved for food contact surfaces. When first approved, a post-rinsing was required, though since 2018, the Danish Ministry of Environment has approved Inspexx 210 DR with no rinse claims. The disinfection agent is a biocide consisting primarily of peracetic acid and peroctanoic acid as well as acetic acid, octanoic acid, and hydrogen peroxide. Inspexx concentrate has a pH-value of 2.4 though the diluted solution had a pH-value of 3.5 and a peracetic acid level at 300 ppm.

Inspexx is an in-process disinfection method and considered an alternative to the widely used method of applying 82°C water. The method requires a contact time of 1-2 seconds at approx. 15°C to obtain the same standard of disinfection as water at 82°C. This enables the room and equipment temperature of the facility to remain low while decreasing the amount of condensation and saving energy of heating water.

On a microbiological level, the acid composition has two main purposes. 1) Peracids affect and destroy different parts of the microorganism including the cell membrane and DNA. 2) Peroctanoic acid affects the phospholipid-bilayer of the cell membrane, which speeds up the penetration of peracids to reach cell DNA.

The acidic composition is claimed to have no effect on the sensory perception like taste, smell, or texture. Also, to not have a toxicological hazard despite not applying a post-rinse with cold water.

Effect

DTI has evaluated Inspexx and compared the effect to disinfection by 82°C water (Rasmussen & Christensen, 2014b). The test was carried out during disinfection of knifes in between removal of offal. The tests were conducted at a Danish slaughterhouse. Between each carcass, the knife was placed in a disinfection box.

The effect of the disinfection methods was based on visual inspection in relation to unintentional coatings as well as microbiological loads of *E. coli*, *Enterobacteriaceae*, and total aerobic plate count. Results showed an equal and acceptable effect of dipping (0.5 sec.) in 82°C warm water and chemical treatment with Inspexx (1 sec.) and an optimized effect of the chemical treatment when combined with a pre-rinse (1 sec.).

Areas for use

Inspexx can be applied to various steps of the production line though it is expected to be corrosive to all surfaces due to the pH value. Also, it is not documented whether there is a sensory related effect on red meat.

Assessment (advantages and disadvantages)

Advantages:
- Functions at low temperatures
- Fast working disinfection
- Optimised disinfection combined with a pre-rinse
- Manual and automated systems
- Does not require a post-rinse

Disadvantages:
- Requires implementation of equipment and maintenance
- Requires safety assessment
- Requires instruction of operators
- Corrosive effect on contact surfaces
- Undocumented sensory effect on meat and meat products

Hydrogen peroxide (H_2O_2) liquid/steam (mist disinfection)

Principle

Hydrogen peroxide can be used for disinfection of surfaces. Using H_2O_2 spray for disinfection of closed rooms H_2O_2 is reduced to water and oxygen in a short time.

Hydrogen peroxide works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components. Catalase, produced by aerobic organisms and facultative anaerobes that possess cytochrome systems, can protect cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen.

Effect

The effectiveness and the potential uses are described in the literature (CDC, 2016).

CDC (2016) describes hydrogen peroxide as active against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and spores. A 0.5% hydrogen peroxide demonstrated bactericidal and virucidal activity in 1 minute and mycobactericidal and fungicidal activity in 5 minutes. Organisms with high cellular catalase activity (e.g., *S. aureus*, *S. marcescens*, and *Proteus mirabilis*) required 30–60 minutes of exposure to 0.6% hydrogen peroxide for a 10^8 reduction in cell counts, whereas organisms with lower catalase activity (e.g., *E. coli*, *Streptococcus* species, and *Pseudomonas* species) required only 15 minutes' exposure.

In an investigation of 3%, 10%, and 15% hydrogen peroxide for reducing bacterial populations, a complete kill of 10^6 spores (i.e., *Bacillus* species) occurred with a 10% concentration and a 60-minute exposure time. A 3% concentration for 150 minutes killed 10^6 spores in six of seven exposure trials. A 10% hydrogen peroxide solution resulted in a 10^3 decrease in *B. atrophaeus* spores, and a $\geq 10^5$ decrease when tested against 13 other pathogens in 30 minutes at 20°C. A 7% stabilised hydrogen peroxide proved to be sporicidal (6 hours of exposure), mycobactericidal (20 minutes), fungicidal (5 minutes) at full strength, virucidal (5 minutes) and bactericidal (3 minutes) at a 1:16 dilution when a quantitative carrier test was used.

Under normal conditions, hydrogen peroxide is extremely stable when stored properly (e.g., in dark containers). The decomposition or loss of potency in small containers is less than 2% per year at ambient temperatures.

H_2O_2 has not been tested at DTI.

Areas for use

Disinfection of clean surfaces:
- High level disinfectant claim: Use 7.5% solution for 30 minutes at 20°C.
- Sterilisation claim: 6 hours at 20°C.

Assessment (advantages and disadvantages)

Advantages:
- May enhance removal of organic matter and organisms
- No disposal issues
- No odour or irritation issues
- Does not coagulate blood or fix tissues to surfaces
- Inactivates *Cryptosporidium* (parasite)

Disadvantages:
- Use as steam/mist disinfection only in closed room including ventilation
- People cannot stay in the room during mist disinfection

Ozonated water

Principle

Ozone is a powerful oxidising agent. It is a more effective disinfectant than either chlorine or chlorine dioxide. It destroys bacteria, yeasts, moulds, and parasites and is most effective at pH 6.0-8.5.

Two mechanisms are proposed to explain the effects of ozone as an antimicrobial agent: Direct interaction of molecular ozone (O_3) with aqueous system components (first order with high redox potential reactions) or free radical-mediated activity. The effect of ozone on microorganisms is dependent on the amount of organic matter surrounding the bacterial cells. Some spores are resistant to ozone, and some are susceptible. Microorganism inactivation is claimed to occur due to the damage to a cell envelope or its disintegration, which leads to subsequent leakage of cellular contents and cell lysis (see Figure 2).

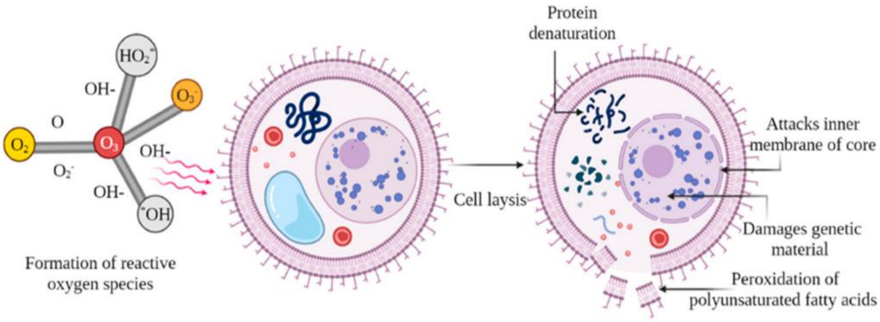

Figure 2. Schematic presentation of decontamination using ozone (Roobab et al., 2023). https://www.frontiersin.org/articles/10.3389/fsufs.2023.1007967/full

Effect

Ozone can reduce the number of bacteria on surfaces (metal, glass, plastic). Holah (2003) has suggested that a rule of thumb is that 2 log reduction can be obtained using 2 ppm ozone for 2 hours. The effect is best at high %RH. Nicolas et al. (2013) showed less than 1 log reduction of *L. monocytogenes* on stainless steel surfaces using 2, 5 or 10 ppm ozone for 1 hour. Using 45 ppm for 1 hour increased the effect to 3.4 log reduction on steel surfaces, but only 0.6 log reduction when the bacteria was bound in biofilms. On plastic surfaces, the reduction was 1.1 and 0.9 log, respectively (cf. Rasmussen, 2015).

Hansen (2000) found no significant reduction of *E. coli* on the surfaces of pork filet (pork loin with rind) treated with up to 5-6 ppm for 1 hour.

Ozone treatment of water containing blood and fat from the slaughter line reduced the total aerobic count by 1-2 log cfu/ml (Sørensen & Granly Koch, 2022).

Areas for use

Useful for elimination of unwanted odour and for cleaning drinking water.

Examples of solutions for air and surface treatment:

- JIMCO A/S (DK) – equipment for combined treatment with ozone and UV-C

- Absolute Ozone (Canada) – example of carcasses treated with ozonated water
- Ozone Solutions (US) – ozone chambers, standard and specially built
- Evergreen Techno Plant (Italy)

Legislation:
Ozone generated from oxygen is on the article 95-list of accepted biocides. No information on acceptable dosage was found after contact to different Danish authorities. MST answered: (Da aktivstoffet endnu ikke er godkendt som aktivstof, er et sådant produkt endnu ikke godkendelsespligtigt og dermed heller ikke vurderet af os. Det er derfor desværre ikke noget, vi kan hjælpe med på nuværende tidspunkt.) As the active substance has not yet been approved as an active substance, such a product is not yet subject to approval and therefore not assessed by us. Unfortunately, it is therefore not something we can help with at this time.

USA: FDA has approved the use of ozone as an antimicrobial agent for direct contact with all foods (June 26, 2001). The United States Department of Agriculture has accepted ozone as an antimicrobial agent for direct contact with meat, poultry, fish, molluscs, and crustaceans (December 2001). Ozone has had USDA-GRAS status since 2002 for the disinfection of meat, poultry and egg products (FSIS directive 7120.1 rev 12).

Japan: In 1996, the Japanese government allowed the use of ozone in direct contact with all types of food.

Canada: The Canadian Food Inspection Agency (CFIA) has approved the use of ozone for cleaning food contact surfaces.

Australia: In 1996, the Australian government approved the use of ozone for contact with all foods.

Assessment (advantages and disadvantages)

Advantages:
Has antimicrobial activity but the effect differs a lot on steel and plastic and on surface bound or biofilm bound bacteria.

A 1-2 log reduction can be obtained in process water from the slaughter line.

Very reactive – is quickly reduced to non-harmful components in aqueous solution.

Disadvantages:
Treatment of surfaces is time consuming.

Avoid contact to products due to oxidation (vitamin, volatiles, colour, firmness).

Toxic to humans in low concentration in the air (50 ppm for half an hour can be deadly). Effective ventilation is needed if used.

Verification

Bactiscan

Principle

EIT International states that bacteria are protected by a protein shell called a surface layer or S-layer, and this shell enables Bactiscan to detect it. The Bactiscan merges four separate wavelengths (UV-A spectrum for user safety) at the fringes of the UV spectrum. The UV bounces off the protein shells and creates a green glow – and this is how unwanted contaminants can be identified. The wavelength differs from traditional UV lamps. It is claimed to measure down to 75 bacteria/gram, and the system has been validated by Campden BRI.

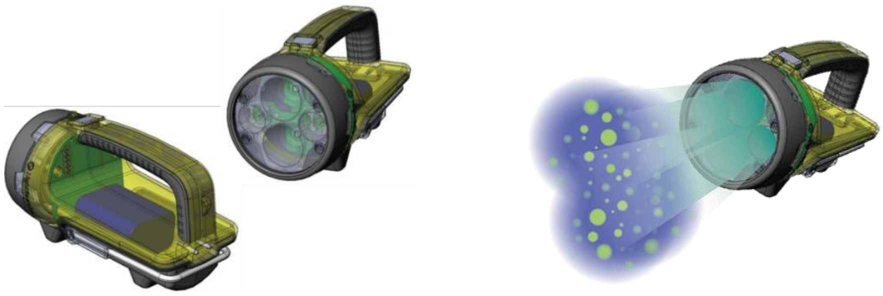

Figure 3. Bactiscan UV-lamp.

Looking into the literature on the bacterial cell, several papers describe the S-layer proteins in bacteria. In Gram-negative bacteria, the S-layer is directly attached to the outer membrane. In Gram-positive bacteria, the S-layer is attached to the peptidoglycan layer.

Gerbino et al. (2015) describe that S-layers are paracrystalline bidimensional arrays of proteins or glycoproteins that overlay the cell surface of several genus and species of bacteria and archaea. As the outermost layer of several genus and species of microorganisms, S-layer proteins (SLP) are in direct contact with the bacterial environment and may thus be involved in many of their surface properties, including adherence to various substrates, mucins, and eukaryotic cells, aggregation and coaggregation with yeasts and other bacteria. In addition, SLP have been reported to be responsible for the bacterial protection against detrimental environmental conditions and to play an important role in surface recognition or as carriers of virulence factors. Bacterial SLP play a role on:

(A) bacterial adherence to different substrates and surfaces
(B) as mechanical barriers in bacterial harmful environments

Sára and Sleytr (2000) concluded that S-layers are the most common cell surface components of prokaryotic organisms. Although structurally diverse S-layers with barely any sequence identities in the constituent subunits are observed even among strains of the same species, these proteins must have domains with common structural and functional significance. S-layer proteins of strains of the same species can be either glycosylated or not. Further they wrote that S-layers of gram-positive and gram-negative bacteria can be lost in the course of subculturing in the laboratory.

Biofilm is defined as a structured community of microbial cells firmly attached to a surface and embedded in a matrix composed of extracellular polymeric substances (EPS). The EPS consist of exopolysaccharides, nucleic acids (extracellular DNA and -RNA), proteins, lipids, and other biomolecules (Karygianni et al., 2020). Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation. These extracellular polymeric substances (EPSs) consist primarily of polysaccharides and can be detected microscopically and by chemical analysis. EPSs provide the matrix or structure for the biofilm. They are highly hydrated (98% water) and tenaciously bound to the underlying surface. The structure of the biofilm is not a mere homogeneous monolayer of slime but is heterogeneous, both in space and over time, with “water channels” that allow transport of essential nutrients and oxygen to the cells growing within the biofilm (Donlan, 2001).

Research has also focused on developing sensors detecting natural fluorescence in bacteria. One example is from Fischer, M. and M. Wahl (2012) who developed an optical fibre-based biofilm sensor to be applied in natural aquatic environments for on-line, in situ, and non-destructive monitoring of large-area biofilms. The device is based on the detection of the natural fluorescence of microorganisms constituting the biofilm. Basically, the intrinsic fluorescence of the amino acid tryptophan is excited at a wavelength of λ=280 nm and detected at λ=350 nm utilizing a numerically optimised sensor head equipped with a UV-LED light source and optical fibre bundles for efficient fluorescence light collection.

Stoica (2018) looked into how spectroscopic techniques combined with chemometric modelling techniques may be used to achieve a solution for a non-destructive – preferably real-time – monitoring system over the hygiene and microbiological level of different surfaces involved in different production processes. More specific, the scenario of monitoring hygienic status of conveyor belts under slaughterhouse processing conditions. An algorithm based on Wilks ratio statistics applied on fluorescence recordings was developed and demonstrated in pilot-scale for its potential to monitor online hygienic status of conveyor surfaces. Strong background interferences and changes in surface physical properties due to processing conditions were identified as the main challenges. The algorithm is designed to neutralise such interference, and a high potential for reaching a valid monitoring solution is expected when using more advanced fluorescence spectrophotometers.

Based on the homepage of EIT International and knowledge from the literature, it is difficult to conclude if the UV-system from Bactiscan measures S-layer proteins (SLP) on the surface of bacteria or if it measures extracellular polymeric substances (EPS) from the microbial built biofilm or other fluorescence compounds in the bacterial cells or food matrices. However, as S-layer proteins are not easy to work with during cultivation of bacteria in the laboratory it might be something else than S-layers that the Bactiscan instrument measures. But most probably the instrument measures EPS or other fluorescence compounds in the biofilm. This would make it more understandable that as low as 75 bacteria/g can be detected as these bacteria might be survivors in a biofilm built for a long time by more bacteria.

Effect

EIT International claims that Bactiscan enables the identification of biofilm, moulds, and bacteria such as salmonella and E. coli. These contaminants are often undetectable using other methods such as white light and UV lamps. With Bactiscan, EIT International states that it is possible to light up the entire surface and obtain an immediate result, so within seconds it is possible to pick things up that would take hours with any other method.

There is no description of how close the lamp and biofilm must be and no information on whether it works in daylight or only in the dark.

Tests at DTI (Bildsted Petersen & Lüthje, 2021) showed that the Bactiscan lamp offers several advantages. It is very user-friendly and works in well-lid rooms. When inspecting large production sites after cleaning, food residues in corners and below equipment can easily be overlooked. With the Bactiscan lamp, organic residues become much easier to detect, and inspectors will thus be less likely to overlook insufficient cleaning.

The fluorescence also makes it easier to take pictures of the soiled areas and thus communicate to the cleaning staff where the trouble areas can be found. Bactiscan also offers a camera that can be attached directly to the lamp.

Though the Bactiscan lamp seems a useful tool during cleaning inspections, there does not appear to be a correlation between fluorescence and bacterial plate count. Thus, the Bactiscan will be very helpful in discovering areas with insufficient cleaning, but fluorescence cannot be directly translated to bacterial numbers.

Areas for use

A good tool for inspection of cleaning e.g., siloes, open plants, and other areas. But there is no correlation to bacterial numbers.

Assessment (advantages and disadvantages)

Advantages:

- Bactiscan makes the cleaning job much faster and therefore more cost effective
- Bactiscan can be used to look at large surfaces quickly
- In most instances, downtime is not required as long as the surface can be illuminated by Bactiscan, for example is Bactiscan able to examine an empty silo in approximately 30 minutes
- No special training is needed to use Bactiscan. Simply point and shoot Bactiscan at the area you want to test

- TekniClean estimates that 10% water reduction might be possible if the last step of disinfection can be avoided or only used where bacteria is located.

Disadvantages:
- No relation to microbial number
- It is difficult to conclude what the Bactiscan measures
- Fresh meat and blood were not detectable in a white plastic box

Safety:
- The company recommends that the inspector wears adequate eye protection in the form of protective glasses whilst using Bactiscan. Safety glass lenses made of polycarbonate will naturally block 99.9% of UV light.

CytoQuant®

Principle

The technic is based on flow cytometry. The CytoQuant® mobile flow cytometer enables the immediate, on-site verification of cleaning procedures in food production facilities or other areas where hygiene is crucial by directly quantifying bacteria and residues on surfaces.

Impedance flow cytometry is a technology that measures cell and particle characteristics with electrical impedance as the method of detection.

The sample is pumped through a microfluidic flow cell with integrated electrodes. An object flowing between the electrodes will introduce a change to the electrical current.

Bacteria have unique electrical properties. Because of their size, the non-conductivity of their cell membrane, and the conductivity of their cytoplasm, bacteria leave a fingerprint that distinguishes them from other particles. This enables CytoQuant® to detect and differentiate between intact cells and particles in a sample.

Figure 4. https://www.romerlabs.com/en/shop/cytoquant-r-flow-cytometer/

For low bacterial numbers, the measurements of the same sample can be prolonged e.g., three times as this will allow detection of a low number of bacteria.

Effect

Measure intact cells and particles in a sample.

CytoQuant® has been tested in the pilot plant facilities at DTI on clean surfaces with and without soap residues, and on surfaces with meat and bacteria. The results have been compared to the traditional swab samples (cfu/cm^2). There was no correlation between CFU/cm^2 and the CytoQuant® results. Furthermore, the detection limit for CytoQuant® at 450 cfu/cm^2 (30 seconds analysis) and 150 cfu/cm^2 (130 seconds analysis) made it unusable for test of the quality of cleaning and disinfection. For more details se Storgaard (2023).

Areas for use

The CytoQuant® mobile flow cytometer has a broad scope of applications. It can be used to quantify bacteria and residues on surfaces in food production facilities, clean rooms, hospitals, and on any other surface where hygiene is crucial.

Assessment (advantages and disadvantages)

Advantages:

- Measures bacteria and residue concentrations on surfaces
- Counts for each swab (cotton bud) sample are provided in 30 seconds, without the need for pre-treatment, incubation, or chemical reagents. Longer time might be needed at low bacterial numbers
- CytoQuant® is easy to use and does not require a lab or special training
- Results are easily transferred to Excel

Disadvantages:

- Might not be comparable to traditional cell count on agar
- Might be affected by NaCl and cleaning and disinfection residues
- The detection limit is 450 cfu/cm^2 (measurement for 30 seconds)
- The detection limit is 150 cfu/cm^2 (measurement for 130 seconds)

Cleaning with water fit for purpose

The use of other water qualities than drinking water for processes in the food industry is developing. Using other water qualities for parts of cleaning processes can be considered, as a means of saving drinking water. The water sources for such initiatives are broad, as most water can be treated to the quality that is needed for cleaning purposes. Whether this is economically and environmentally beneficial must be evaluated in larger terms. A potential source of water for treatment and reuse as water fit for purpose is process water that has already been used in the production. Another potential source is rainwater. Interest in using water that exceeds the limits of chemical substances in drinking water is also rising.

Principle

The basis of decision making when applying the use of process water for cleaning purposes includes the following activities:

- Cost benefit analysis – business case
  - Water prices, including heating costs
  - Wastewater treatment costs
  - Estimated technology investment incl. distribution and CIP costs
- Theoretical risk analysis (microbial, chemical, physical)
  - Regulatory barriers (legislation, customers)
  - Describe water quality
  - Describe intended use
    - Initial cleaning (floors, conveyers, equipment, stable, etc.)
- Baseline study (microbial, visual, chemical)
  - Water quality from the source process
  - Water quality demand and environment in the place of reuse
- Design the process water treatment (technology partner)
  - Treatment steps to obtain the water quality demanded
  - Distribution system layout
- Evaluate the process
- Microbial analysis of the new process (the surfaces after cleaning)
- Update the risk analysis
- Monitoring water quality
  - How and where?
  - Online measuring versus lab analysis
- Update the cost benefit analysis – business case
- Dialogue with authorities based on risk analysis

Effect

The water treatment train must focus on both microbial, organic and chemical parameters relevant for the specific process for using water fit for purpose.

Some examples and parameters are conductivity, turbidity, pH, temperature, COD or TOC, nitrate, nitrite, phosphorus, aerobic count, E. coli, aerobic spores.

Some examples of water treatment are filtration at different sizes (10^{-3} to 10^{-10}; e.g., GF, BF/CF, MF, UF, NF, RO), UVC, ozone, chemical disinfection, heating etc.

The effect of these technologies will vary and depend on the contamination of the process water and the capacity of the treatment system etc. Some technologies will have only a 1 log reduction of bacteria others up to a 6 log reduction.

Areas for use

Treated process water can be used for pre-rinse. However, care must be taken to document that the use will not affect food safety, shelf life or the quality of the cleaning process (microbial and ATP results). Therefore, a risk analysis must be made in each case describing the water source, storage, and intended use, as part of the basis for decision making.

The quality of the water that is considered fit for purpose must be like potable water unless the company can demonstrate that there is no risk to food safety of the final product, which should be accepted/approved by the competent authority (Europa-Parlamentets og Rådets forordning (EF) nr. 852/2004 af 29. april 2004 om fødevarehygiejne, kap. VII stk. 3).

The Danish legislation on the quality of water used for cleaning is not clear. The legislation states that cleaning and disinfection must ensure that hygiene is acceptable, and food is not contaminated (kap. 19.1, VEJ nr. 9866 af 27. juni 2022, Vejledning om fødevarehygiejne). Furthermore, the Danish legislation states that only water of drinking quality must be used for cleaning in the food industry (Europa-Parlamentets og Rådets forordning (EF) nr. 852/2004 af 29. april 2004 om fødevarehygiejne). Further reading in Appendix 1 (in Danish).

For further information see Koch, A.G., Sørensen, K. & Petersen, E.B. (2022) and Koch (2023).

Assessment (advantages and disadvantages)

Advantages:

- Improve sustainability in the company
- Savings on water usage
- Savings on wastewater volumes for treatment

Disadvantages:

- Technology for water treatment might be costly
- Water from processing must be collected during the day for use in the subsequent cleaning shift
- Transport (tubes) and storages (tank/silo) of water need space in the factory and can be expensive, also cleaning systems for pipes and tubing might be needed

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