A new approach for biologically inhibiting surfaces

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A biologically inhibiting surface based upon an electrochemical principle has shown to be very effective against the formation of biofilm in water containers and supply systems. The coating consists of silver and another precious metal, which is applied to the surface in small areas with a thickness measured in nanometer. Due to the difference in the potentials, the biologically inhibiting material will act as a galvanic element in contact with an electrolyte. But the electrochemical processes taking place at the metal surface seems to be of a catalytic oxidation character more than an oligomeric effect from the silver.

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Introduction

The microbiological activity in e.g. water systems is defined by the planktonic (free-flowing) and sessile (biofilm) organisms in the slime film on surfaces. In practice the number of planktonic cell is the parameter monitored and which should be minimized, while the biofilm is rarely controlled. However, the biofilm is often the cause for sudden increasing number of planktonic cells and for the development of pathogen bacteria. Biofilm offers a natural protection against biocide and cleaning, and therefore cells may survive in the biofilm and later be released to the media.

The use of copper and silver ions to obtain oligomeric effects has been used since the ancient time[1]. This well known property of silver ions[2] has during the last years been introduced into a lot of products such as polymer surfaces, fabrics etc.[3]. For all these products the mode of operation is release of silver ions and numerous patents already cover that topic. The minimum inhibitory concentration of electrically generated silver ions has for some bacterial strains been estimated[4] to 30-125 µg/L, and the necessary concentration for full killing effect (minimum bactericidal concentration) 480-1005 µg/l. The method of silver ions is effective, but in many situations the direct release of chemicals is unwanted.

The substrate material and surface topography[5] also have some effect on the rate of biofilm formation, but no direct inhibiting effect can be found in any substrate material. It has recently been shown that even a solid pure silver surface does not have a significantly lower degree of microbial adhesion as compared to stainless steel[6]. However the properties may change with size. Silver is characterised by good electrical conductivity, high nobility and catalytic properties even in liquid phase catalysis. The latest feature is argued from the use of silver microcrystals immobilized on alumina in filters for cleaning pools[7], where the oxidation at the silver surface is claimed to be useful for destruction of bacteria, viruses and other organic materials. The oxidation process can be improved by using chlorine or ozone in small amounts[8].

Another strategy for reducing biofilm formation is the use of imposed potential or electrical current. Studies[9-12] have shown that biocide efficacy can be improved if oscillating potentials, current or constant potentials are applied, and effects have mainly been explained by local changes in pH, transport effects or generation of oxygen. However, imposing potentials on surfaces requires an external voltage, electrical contacts etc., and is somewhat unpractical.

It is also a fact that cell membranes become more permeable in an electrical field. This is well known from electroporation in the field of molecular biology. Here an electrical field is applied as a technique and an effective way of introducing different substances inside a cell, such as drugs or more sophisticated a piece of

coding DNA. The effect of the electroporation is theoretically explained as a process that introduces very small openings (pores) in the plasma membrane, which increase the permeability.

It is desirable to modify surfaces to achieve an inhibiting effect on biofilm formation directly on the surface. The inhibiting surface[13] in focus in this paper has been designed to form an electrical field on the surface itself. It is based on silver combined with another noble metal, both with catalytic properties. The surface is micro/nano structured to ensure high local electrical field strength. The surface structure is characterized by the appropriate distribution of the one electrode material in small isolated areas, either as microclusters on the surface of the other electrode material or as micro-holes in the surface of the other electrode material with a proper and not too large distance between adjacent microclusters or micro holes.

The surface may also release small amount of silver ions. But in general this content will be without any oligomeric effect because of very low concentration in the range of a few ppb silver. On the other hand silver complexing compounds in the media, especially cyanide and ammonia, can increase the dissolution of silver.

The mechanism behind the effect will be discussed based upon thermodynamic considerations and experiments carried out in different microbiological media.

Thermodynamic calculations

Anodic polarization of a silver surface in an electrolyte containing only about 40 ppm (less than the content in tap water) will immediately form a very thin layer of adherent AgCl at the surface. The formation of AgCl can easily be explained from thermodynamic calculations given as a Pourbaix diagram in Figure 1.

The chemical reactions on the surface in tap water can convert the pure silver to an insoluble compound at the surface such as AgCl. The release of silver ions from the surface is insignificant because of the low solubility product for AgCl (1.77×10^{-10}) even at potentials over the equilibrium potential for the electrochemical reaction (Eq. 1).

$$AgCl + e- = Ag + Cl^- \qquad \text{Eq. 1}$$

The diagram in fig. 1 is superimposed by the immune area for Pd, showing a potential difference of 200 mV between pure Pd and Ag at pH 7. This shows that

coupling of the two materials will polarize the silver electrode anodically and thereby facilitating the reaction in Eq. 1.

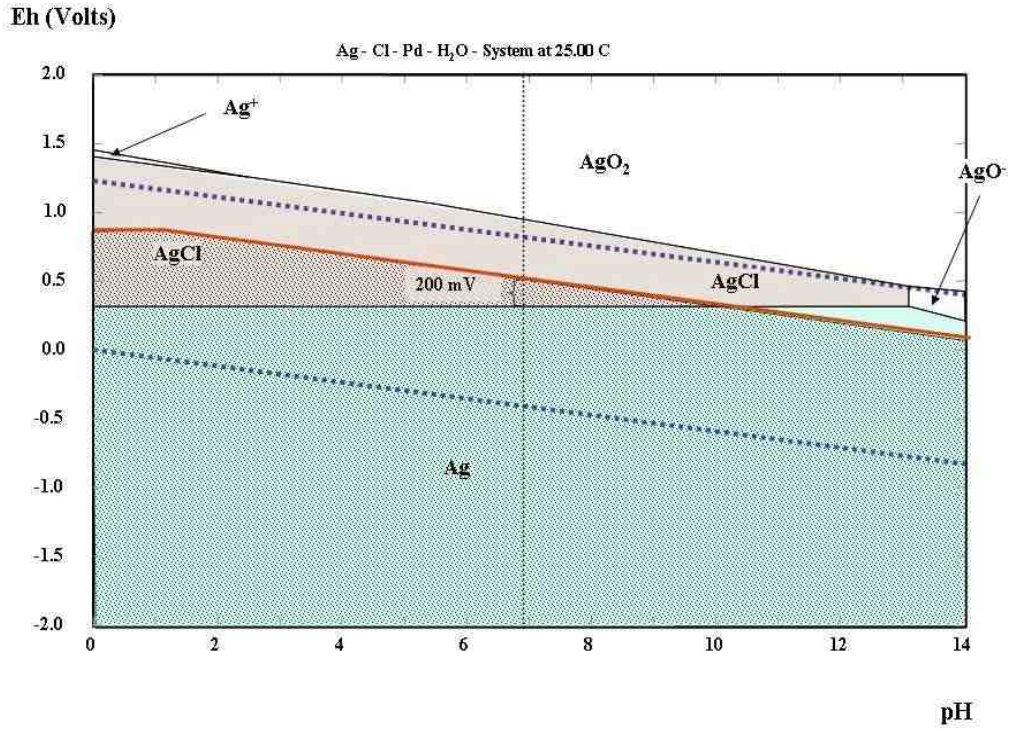

Figure 1. Pourbaix diagram calculated for metal ion concentration 10^{-6} M/l. The green area and the hatched area below the red line (in a large area identical) show respectively the immune area for silver and palladium in a solution containing 0.025 mol Cl^-/l. The brown area shows the thermodynamically stable area for AgCl. Even at very low concentration of chloride (approximately $2 \cdot 10^{-4}$M/l) AgCl is formed.
The diagram shows a potential difference of 200 mV between pure Pd and Ag at pH 7 in the actual case. This explains the formation of AgCl even in water with low chloride concentration such as drinking water, if silver is coupled to palladium.

In the literature[14] it has been stated, that silver ions can be reduced to silver by oxidation of hydroxyl groups in organic compounds. These observations are in good agreement with thermodynamically consideration where ethanol is used for the verifications.

$$4AgCl + C_2H_5OH + OH^- = 4Ag + CH_3COO^- + 4Cl^- + 4H^+ \qquad \text{Eq. 2}$$
$$\Delta G = -117.799 \text{ kJ } (20^oC)$$

Since the Gibbs free energy for the reaction (ΔG) is negative, this reaction (Eq. 2) is thermodynamically favourable.

Furthermore reducing sugars such as fructose, glucose, glyceraldehyde, lactose, arabinose maltose and monosaccharides containing ketone groups can be oxidized of AgCl to CO_2 or to carboxylic groups.

$$24AgCl + C_6H_{12}O_6 + 6OH^- = 24Ag + 6CO_2 + 24Cl^- + 18H^+ \qquad \text{Eq. 3}$$
$$\Delta G = -1016.175 \text{ kJ } (20^oC)$$

From a thermodynamically point of view it is therefore not surprising that organic species can interact in an oxidation process with a AgCl surface, if the kinetics are sufficiently fast.

The regeneration of the silver chloride after the oxidation process of the organic species can be carried out only in the presence of O_2 (aerobic conditions), where silver is easily oxidised in connection to the reduction of oxygen on the Pd cathode – given that the silver material is coupled to palladium.

$$4Ag + 4Cl^- + O_2 + 2H_2O = 4AgCl + 4OH^- \qquad \text{Eq. 4}$$

This reaction can be separated in an anodic and a cathodic partial reaction:

$$4Ag + 4Cl^- = 4AgCl + 4e^- \text{ (anodic partial reaction)} \qquad \text{Eq. 5}$$

$$2H_2O + O_2 + 4e^- = 4OH^- \text{ (cathodic partial reaction)} \qquad \text{Eq. 6}$$

Later experiments in this paper (fig. 5) seems to confirm that organic species such as aldehyde groups can be oxidized on the silver surface, and a corresponding reduction of oxygen or another specie can take place on the palladium surface.

Materials and methods

Design of surface

The active surface on the biologically inhibiting material is silver which has been surface treated by immersion plating in an incomplete deposition process, so that the top electrode material palladium only covers the bottom material partially exposing the bottom electrode material in microholes. The surface structure is obtained by conventional electroplating of silver and conventional immersion of palladium as described in the patent[13].

For the electrochemical test and the test in milk (see below) stainless steel AISI 316 rod or sheet is used as base material on top of which an interlayer of Wood nickel is electroplated, followed by electroplated technical silver. This silver surface is then immersion plated with palladium. In the other tests given in this paper solid technical silver (99.75%) material in the form of 0.5 mm sheet or 0.5 mm diameter wire is used as base material. For the Pd plating from $PdCl_4^{2-}$ (Eq. 7, given later) a 5 % solution of the stock solution (0.5 g/l $PdCl_2$ and 4 g/l NaCl dissolved in water) is used with 3 minutes contact time at ambient temperature.

Final control of surfaces is visualization in scanning electron microscope (SEM) and analysis by energy dispersive x-ray (EDX) at 10 kV.

As reference material stainless steel AISI 316L, 2B finish, pickled has been used in the form of sheet of 1 mm thickness.

Electrochemical test
The purpose of this test is to examine whether a coupling of the two catalytical metal surfaces (silver and palladium) can electrochemically degrade an organic compound containing chemical groups that can be oxidized or reduced. The setup is therefore designed to measure the faradaic current possibly introduced by addition of an organic compound to a coupling of silver and palladium in an electrolyte.

In a standard glass corrosion cell of 400 ml volume cell two electrodes are connected through a potentiostat as zero resistance ammeter ZRA. The galvanic current between the silver plated AISI 316 stainless steel sample of 3.93 cm^2 and a palladium electrode of 1.93 cm^2 is measured. The test solution is 0.5 M sodium acetate buffer at pH 6, including 3% NaCl, kept at 22 ºC. The solution is stirred and in contact with air. The experiment runs for 48 hours. After stabilization of the current, 0.5 g of paraformaldehyde HCHO is added to the test solution. This is to investigate, if this organic matter as a model system for bacteria can be oxidized or reduced on the electrodes, resulting in an increased current measured.

Biological test setups
The focus of the biological tests is twofold: passing a media of living cells over the inhibiting surface to investigate the effect of contacting free-flowing cells, and the effect on direct biofilm formation of the inhibiting surface as compared to stainless steel 316L, 2B finish, pickled. For this paper results from 3 different demonstration experiments have been selected.

Tube and wire test with circulating media
A 200 ml flask is connected with a silicone rubber tube and a pump to assure circulation of the solution in a closed system. A silver palladium wire is

incorporated in the silicone tubes and in a reference setup this wire is excluded. The wire is coiled with an inner diameter of 5 mm, and a total surface area of 80 cm^2 per L TSB (tryptic soja broth) solution giving a surface to volume (S/V) ratio of 0.08 m^{-1}. The flow is 60 ml/min and the experiment is run at 30°C. A high (10^6 CFU/ml) or a low (10^3 CFU/ml) level of *E. coli* (28.0017) bacteria is added and the system is allowed to run for 24 hours. The bacterial number as CFU/ml is determined by dilution and incubation of 1 ml samples from 0, 3, 6 and 24 hours on TSB plates.

Test in milk

18 metal plate specimen of 25·10·1 mm dimension (exposed surface area of each 3.6 cm^2) are placed through the top lid in 1.4 l volume polypropylene (PP) cylindrical reactors. The S/V ratio is 0.05 m^{-1}. In one reactor the metal samples are stainless steel AISI 316L, 2B finish, pickled and in the other reactors similar sheet specimen have been surface treated to have a silver palladium surface. The reactors are filled with milk and the temperature is kept at 21 °C under good circulation. *E. coli* K12 bacteria are inoculated to give an approximate level of 10^4 CFU/ml. Samples of milk is taken each hour for the first 6 hours and after 24 hours for estimation of cell number. Initial biofilm formation is examined after 24 hours by confocal laser scanning microscopy (CLSM) on metal samples after coloration for protein and fat.

Test in natural drinking water

In PP cylindrical reactors (fig. 2) of 1 L volume a number of tests are performed with poor quality natural drinking water (approx. 400 CFU/ml of naturally occurring cells).

Figure 2. Polypropylene reactors (left) and sample holder (right) for metal specimens.

Two reactors include 18 stainless steel reference samples each, while the other reactors each include 13 silver palladium samples and 5 stainless steel samples. The S/V ratio is 0.05 m^{-1} for the silver palladium containing reactors. Tests are run with new silver palladium surfaces and with used silver palladium surfaces, which have been pre-exposed in natural drinking water for 4 weeks and then autoclaved to kill possible present bacteria. The experiment is performed in duplicate.

The experiment runs at 15°C with good stirring. Twice a week 1/5 of the volume is exchanged with fresh tap water, but no nutrients are added during the 28 day experiment. Two different growth media are used for the estimation of bacterial numbers by Heterotrophic Plate Count (HPC): one based on yeast extract, which is normally used according to Danish standard DS 6222, and one denoted R_2A which is more sensitive to the types of bacteria present in natural drinking water, but which requires 14 days of incubation. Metal samples are taken out for biofilm estimation at day 7 and 28. The biofilm is sampled by swapping. Silver analysis of the water phase is carried out by ICP-MS for samples from days 10 and 28 for one of the reactors containing stainless steel only and for one of the reactors containing new silver palladium samples.

Experimental results

Design of surface

The desired structure is given schematically in fig. 3. It is important to obtain separation of the two metals and contact of both to the media and thus the bacteria.

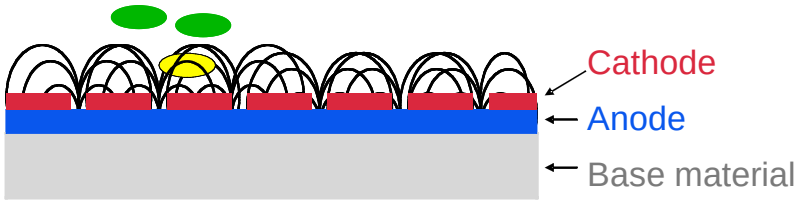

Figure 3. Schematic showing the micro/nano structured surface design. Discrete areas of materials of different nobility is designed with less than 50 µm distance generating locally high electrical fields to inhibit bacteria contacting the surface. Green spots illustrate live cells and the yellow spot a live cell inhibited.

The obtained surface structure is shown in fig. 4 as a SEM micrograph. Analysis of the composition by EDX at 10 kV gives in weight % approximately 97 % Ag and 2.5 % Pd in the darker areas, and 86 % Ag, 12 % Cl, and 1.5 % Pd in the light nodules.

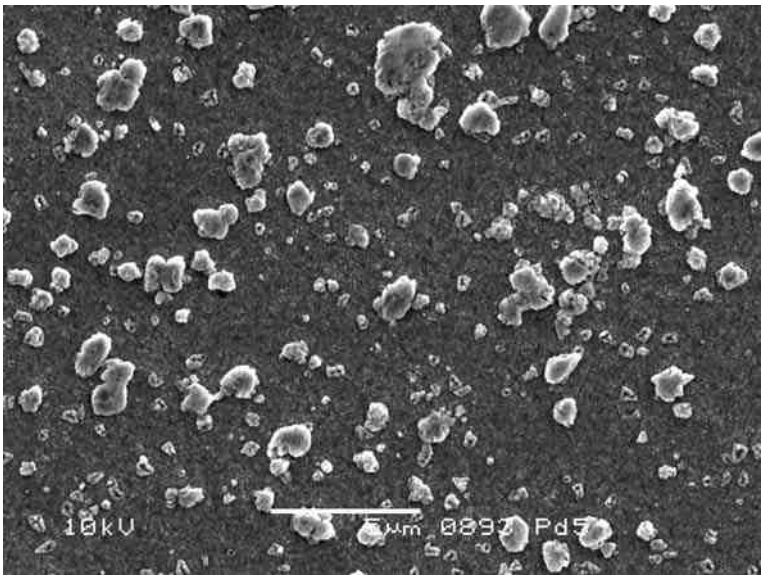

Figure 4. SEM micrographs showing the micro/nano structured surface design. Light nodules are silver chlorides formed on exposed silver areas, while darker area is silver covered with a few nanometer thin palladium layer.

Palladium is deposited in a very thin layer with microholes on top of silver. In the microholes silver has reacted during the deposition of palladium to form small clusters of silver chlorides visible as light nodules. The obtained distance between discrete areas is less than 5 µm. The reaction given AgCl during deposition is given below (Eq. 7).

$$PdCl_4^{-2} + 2Ag = 2AgCl + Pd + 2Cl^- \qquad \text{Eq. 7}$$

Electrochemical test

The result of measuring the galvanic current between a silver electrode and a palladium is given in fig. 5. The initial anodic current measured is most likely due to AgCl being developed on the silver surface, as the coupling with palladium will increase the potential. The area ratio is 2:1 for silver vs. palladium. As seen on the graph the DC current is leveling out adjusting towards a steady state of approximately 0.009 µA (0.005 µA/cm^2 for the silver surface) after 35 hours. After 45 hours test 0.5 g paraformaldehyde is added. The current immediately increases by 0.008 µA and stays at the higher values of 0.018 µA for the remaining 3 hours of the test.

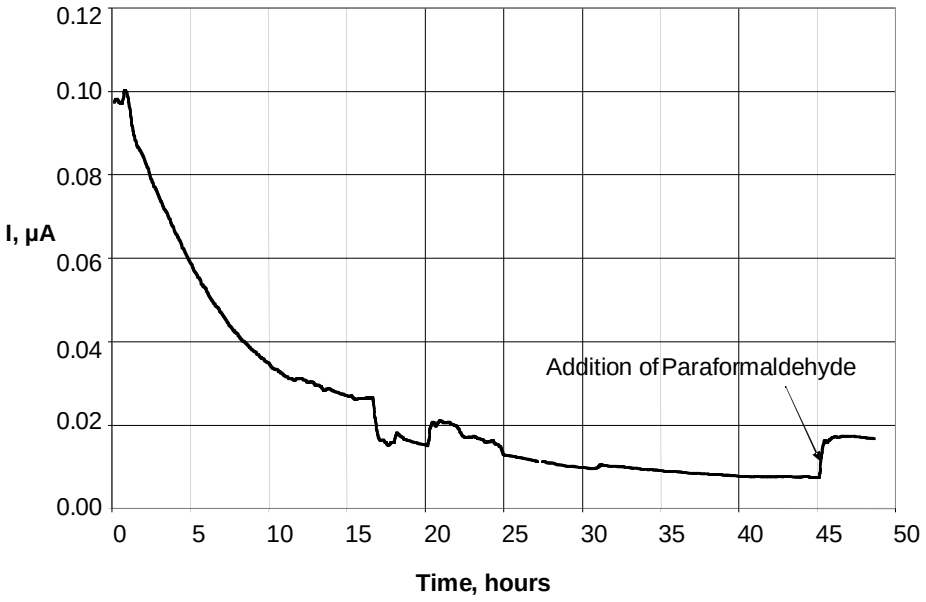

Figure 5. Galvanic current measured between silver and palladium in pH 6, saline solution, before and after addition of 0.5 g paraformaldehyde at 45 hours.

It is clear that an increased Faradaic current is observed, when the paraformaldehyde is added to the test solution. This observation may be explained by a number of reactions as given below.

The anodic reaction takes place at the Ag/AgCl surface, where AgCl is reduced of the paraformaldehyde to pure silver while oxidising of the reducing specie to formic acid or formate:

$$2AgCl + HCHO + OH^- = 2Ag + 2Cl^- + HCOO^- + 2H^+ \qquad \text{Eq. 8}$$
$$\Delta G = -127.049 \text{ kJ } (20^oC)$$

The silver surface is in presence of chloride immediately reoxidized by oxygen to AgCl due to the galvanic coupling between the silver and palladium electrode. The palladium electrode acts as the cathode in the electrochemical setup (Eq. 6).

$$4Ag + 4Cl^- + O_2 + 2H_2O = 4AgCl + 4OH^- \qquad \text{Eq. 9}$$
$$\Delta G = -70.943 \text{ kJ } (20^oC)$$

An alternative proposal for the anode reaction giving the increased current could be a directly oxidation of the organic species at the silver surface. In the present

case an oxidation of paraformaldehyde would directly take place upon the silver electrode surface without interaction with any AgCl layers.

$$2HCHO + O_2 = 2HCO_2^- + 2H^+ \qquad \text{Eq. 10}$$
$$\Delta G = -483.964 \text{ kJ } (20^oC)$$

On the other hand the Faradaic current observed in the experiment could also indicate a reduction and oxidation of the present organic species (an aldehyde) by the two electrodes. A reaction of that type is called a Cannizaro reaction:

$$2HCHO + OH^- = CH_3OH + HCOO^- \qquad \text{Eq. 11}$$
$$\Delta G = -149.982 \text{ kJ } (20^oC)$$

The Cannizaro reaction can be divided up in two separate electrode reactions taking place respectively at the cathode (palladium), where paraformaldehyde is reduced to methanol, and at the anode (silver), where paraformaldehyde is oxidized to formic acid or formate.

$$HCHO + 2\,H^+ + e^- = CH_3OH \quad \text{cathode reaction} \qquad \text{Eq. 12}$$

$$HCHO + OH^- = HCOO^- + 2H^+ + 2e^- \quad \text{anode reaction} \qquad \text{Eq. 13}$$

The proposed electrode reactions (Eq 12 and 13) above indicate that a combination of silver and palladium as catalytic metals in an electrolyte also could stimulate a random oxidation and reduction of organic species in absence of the cathodic reduction of oxygen. The only assumption for the occurrence of such type of reactions is the presence of chemical groups, which are active in an electrochemical redox process.

Biological tests

The biological test are conducted with the purpose of examining the effect on both planktonic and biofilm bacteria, and if the proposed inhibiting mechanism of the coating is plausible.

Tube and wire test circulating media

In this test the effect on growth of E. coli in the media was monitored, and no attempt was made to investigate the actual biofilm on the wire surface. Representative results are shown in figures 6 and 7. For the system with high bacterial load (fig. 6), the growth of cells is retarded already after 3 hours and after 6 hours contact the number of cells in the system with silver palladium wire

is close to zero. The measurement after 24 hours however shows that regrowth has appeared, but the total cell number is 4 decades lower than the reference.

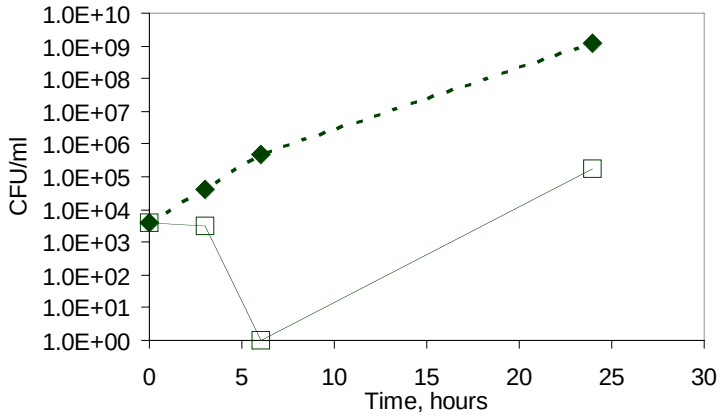

Figure 6. High concentration of E. coli, 30 ºC, circulation in silicone tube with silver palladium coiled wire (□) or in reference silicone tube without metallic material (♦). Note logarithmic scale on y-axis.

For the system with low bacterial load (fig. 7), the number of cells in the system with silver palladium wire is close to zero after just 3 hours and no regrowth appears. The reference system shows a growth pattern similar to the reference in fig 6, though starting from lower values.

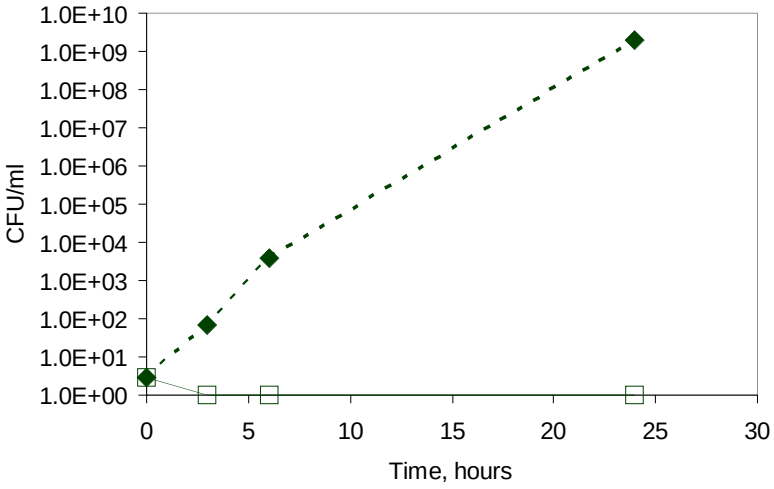

Figure 7. Low concentration of E. coli, 30 ºC, circulation in silicone tube with silver palladium coiled wire (□) or in reference silicone tube without metallic material (♦). Note logarithmic scale on y-axis.

Test in milk

The data in fig. 8 shows the number of E. coli in milk in the two containers including silver palladium coated stainless steel plates or stainless steel plates. There is no difference in growth pattern between the two systems for the first 6 hours, but after 24 hours growth has appeared in the reference system and not in the test system. At this point there is a 3 decade difference in cell number.

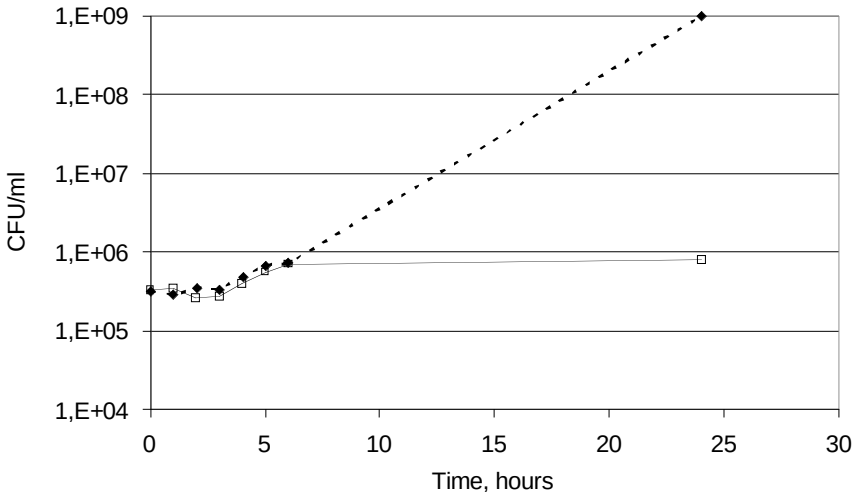

Figure 8. Milk, 21 ºC, E. coli addition. One reactor includes plates coated with silver palladium (□) and the reference includes stainless steel (♦). Growth is inhibited in the reactor with silver palladium. Note logarithmic scale on y-axis.

Metal samples from the milk reactors were used for checking biofilm formation by CLSM fluorescence technique (fig. 9). The tests with silver palladium coated surfaces all show no reaction with the active colors, indicating no presence of protein or fat on these surfaces. Thus no initial biofilm formation could be detected after 24 hours. On the stainless steel controls, however, protein and fat could be detected indicating that a conditioning film had been formed within 24 hours.

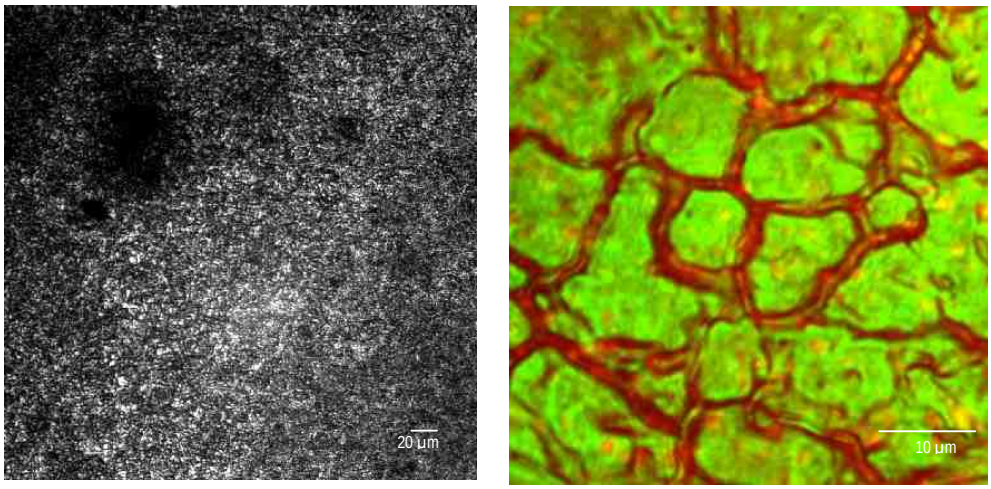

Fig. 9 CLSM. Right: Reflection mode silver palladium. No reaction could be found in fluorescence mode on this surface. Left: fluorescence mode picture of stainless steel with protein (green) and fat (red). Parallel tests with fat only gave similar results of no reaction on silver palladium, but clear presence of fat in especially grain boundaries on stainless steel.

Test in natural drinking water

In natural drinking water the presence of bacteria should be very low, and the available nutrients very limited. However, the chosen tap water is of poor quality with bacterial numbers exceeding the limit values.

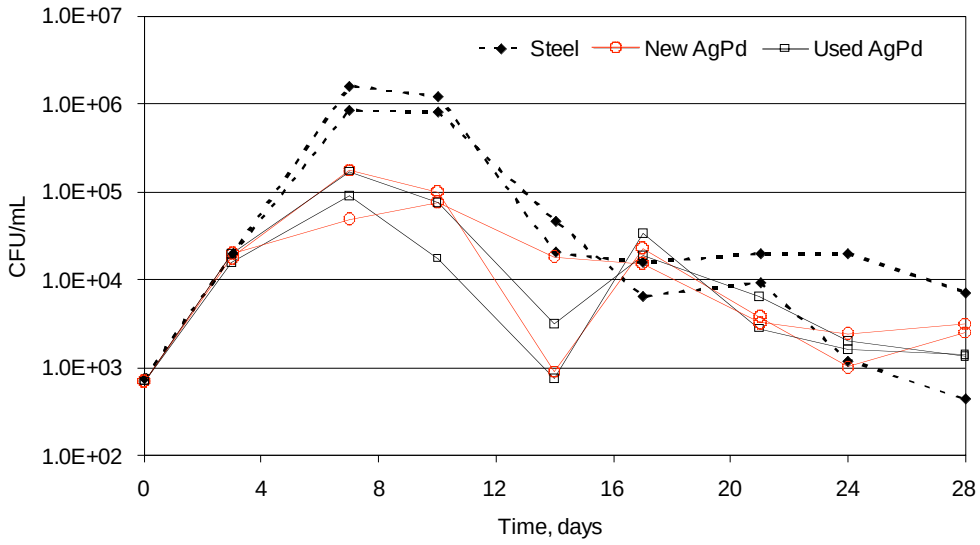

Figure 10. CFU/ml in natural drinking water in contact with three different surface types, stainless steel (♦), new silver palladium (o) and used silver palladium (□). The tests are duplicated. Yeast extract is used for the enumeration procedure according to Danish standard for drinking water. Note logarithmic scale on y-axis.

The test results from the reactor experiments given in fig. 10 and 11 illustrate that growth of planktonic cells do occur in this semi-batch system (1/5 volume exchanged twice a week) but that the number of cells decreases again within the 4 week experiment. No nutrient is added, but it may be expected that the PP material releases small amounts of biodegradable compounds.

The comparison of reactors with stainless steel as opposed to silver palladium shows a higher cell number for stainless steel. The new and used silver palladium surfaces give a significant lower number of cells. The tendencies are the same whether yeast extract (fig.10) or the R_2A (fig. 11) growth media is used in the enumeration procedure.

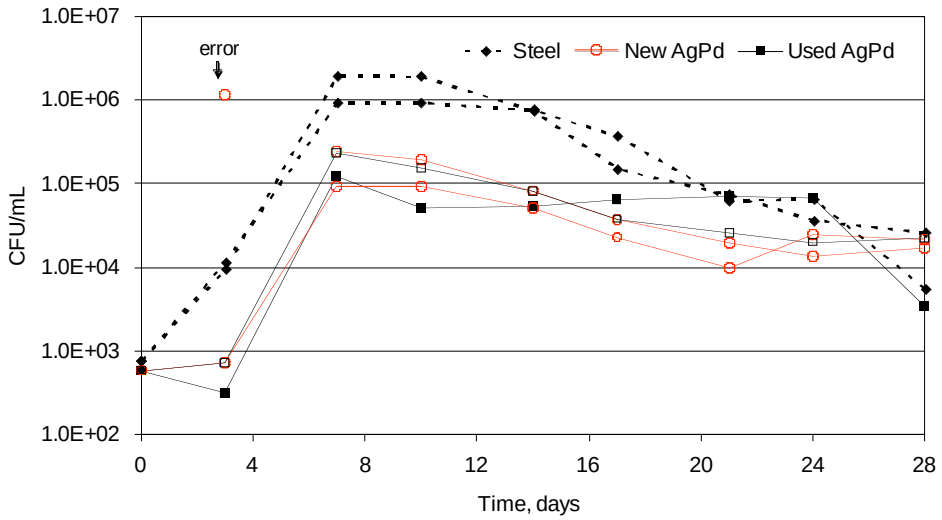

Figure 11. CFU/ml in natural drinking water in contact with three different surface types. The tests are duplicated. R_2A media is used for the enumeration procedure giving good conditions for growth of naturally occurring drinking water bacteria. Note logarithmic scale on y-axis.

In fig. 12 the development in presence of biofilm on the surfaces is shown. It is evident that after 7 days the amount of biofilm on stainless steel surfaces is substantial as compared to all silver palladium surfaces, where only negligible amounts can be detected. After 28 days the amount of biofilm is very low and almost equal on all surfaces. At this point the number of planktonic cells has also decreased.

Analysis of the water phase at days 10 and 28 gives below 1 µg/l silver in the systems with new silver palladium and approximately 1 µg/l in the stainless steel

system. This is the detection limit for the ICP-MS system, and also values too low to indicate any toxic effect of silver ions.

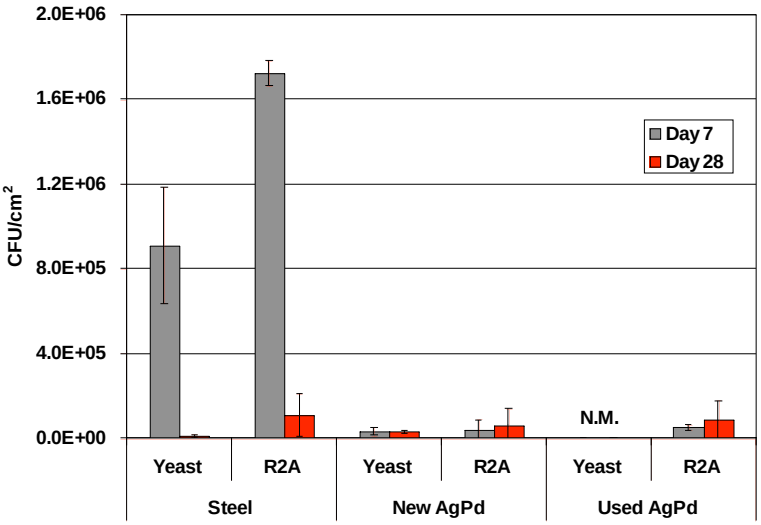

Figure 12. Biofilm estimation from metal samples in natural drinking water, days 7 and 28. Three different types of surfaces were analyzed with two different growth conditions (yeast and R_2A) during enumeration. Stainless steel samples showed highest degree of biofilm formation after 7 days. Note linear scale on y-axis.

The results obtained in natural drinking water show a strong inhibiting effect on the biofilm formation after 7 days and a significant reduction of planktonic cells for the silver palladium surfaces as compared to stainless steel, - without the release of silver ions. The effect is approximately one decade reduction in CFU/ml as compared to stainless steel. This effect is obtained with the limited surface area available (S/V ratio of 0.05 m^{-1}).

Summary and conclusion

The desired surface structure[13] illustrated in fig. 3 can be designed with conventional techniques. It has been shown that silver coatings applied to stainless steel (or non-conducting materials such as ceramic and polymers) can be micro/nano structured by treatment with metals such as Pd, Ru or Pt for formation of small catalytic areas, where a cathodic reaction can take place. The remaining surface consists of silver and act as the anode, where organic species such as living cells or protein/fat can be oxidized. The silver coating can easily be applied with electroplating on the top of stainless steel after a conventional activation of the surface or by PVD or electroless silver plating on the top of non-conducting

materials. The catalytic Pd metal can be easily applied by an immersion process using a tetrachloropalladate(II)-2 ion ($PdCl_4^{-2}$).

It has been shown that these surfaces have an inhibiting effect on planktonic cells in a media contacting the surface. It has further been shown that biofilm formation is delayed on these surfaces or directly inhibited. However, the specific media, the organic load and the flow pattern are important parameters determining the effectiveness of the surface. The experiments show a significant effect on elimination of biofilm and killing of bacteria in tap water.

The oligomeric property of silver cannot explain the inhibiting effect found in the results. The concentration of silver ions has been measured to be under 10 ppb in several practical experiments, e.g. tap water, documenting that silver ions in this low concentration cannot be responsible for the inhibiting effect. To obtain oligomeric effect of silver a minimum content of 30-125 ppb is necessary[4]. The observed results are in good agreement with the solubility product of AgCl in a solution like tap water.

It is therefore evident that the effect cannot be explained from the inhibiting effect of silver ions alone– but probably more from the electrochemical interaction or electric field between the two catalytic metals palladium and silver and the organic species.

As shown by thermodynamic calculations the presence of chloride even in small quantities (about 10 mg/l) will promote the formation of an adherent AgCl film. The formed AgCl film does not seem to inhibit the oxidation/destruction of organic species or the killing of microorganisms on the surface.

Thermodynamic considerations show that the oxidation of selected organic species is possible both at the AgCl surface and at a pure silver surface. It has also been found (fig. 9) that the coating is able to eliminate the immobilizing of proteins and fat at the surface. The organic material is probably oxidized away.

The mechanism for killing bacteria at the surface is in the moment not fully explained. The concentration of silver ions cannot explain the mechanism in general. However, it cannot be excluded, that the effect can be partly explained by an electroporation process, where the plasma membrane in the living cell is harmed by the electric field caused by the redox processes, which causes an increase in the permeability of the cell membrane, thus making the cells susceptible to very low silver concentrations.

Acknowlegdements
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