Biofouling on plate heat exchangers and the impact of advanced oxidizing technology and ultrasound

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Abstract

Fouling in general and biofouling in specific is a problem concerning many industries. Biofouling occur in environments favorable for biological growth. As an example, industries using cooling water have problems with biofouling. The problem is apparent on heat exchangers as biofilm reduces the heat transfer and thus the performance. There are several different methods to reduce fouling on tube and shell heat exchanger on the market. However, for plate heat exchanger the alternatives are fewer. Generally, cleaning is performed by opening the heat exchanger and cleaning the plates one by one.

This thesis aimed to present some of the biofouling reducing methods available on the market, and principally methods that could be applied on plate heat exchangers. Two of the methods were selected to be evaluated in experimentally; Advanced Oxidizing Technology (AOT) and ultrasound. The performances were evaluated by semi quantifying the amount of produced biofilm on plates of stainless steel, polystyrene and titanium. The plates were either reference plates, i.e. under no influence of some reducing method, or they were submerged in the tank when ultrasound or AOT were under operation. The test with ultrasound was performed once, whereas the test with AOT was performed twice. Ultrasound showed the best result; by reducing the amounts of produced biofilm from 68-100 % on all the plates, independent of the material used. AOT showed ambiguous results. From the first test it seemed to affect the production of biofilm, whereas in the second test it did not proof to have the same affect.

Sammanfattning

Påväxt i allmänhet och biologisk påväxt i synnerhet är ett problem som finns i många industrier/områden inom industrin. Biofilmstillväxt förekommer i miljöer gynnsam för biologisk tillväxt. Till exempel har industrier som använder kylvatten problem med biofilmstillväxt. På värmeväxlare visar sig problemet i form av sämre effektivitet genom försämrad värmeledningsförmåga. Det finns flera olika metoder för tillväxt att förhindra eller motverka tillväxt i tubvärmeväxlare. För plattvärmeväxlare är alternativen däremot färre. Generellt rengörs värmeväxlaren genom att denna öppnas och att plattorna rengörs en efter en.

Syftet med detta examensarbete var att presentera några av de metoder som finns på marknaden för att minska och ta bort biologisktillväxt, och då främst metoder som går att använda på plattvärmeväxlare. Två av metoderna valdes ut och testades; Advanced Oxidizing Technology (AOT) och ultraljud. Metoderna utvärderades genom en semi- kvantitativ analys av mängd producerad biofilm på plattor gjorda av rostfritt stål, polystyren och titan. Dessa plattor var antingen referensplattor, det vill säga utan inverkan av någon reduceringsmetod, eller så var de nedsänkta i tankar fyllda med vatten där respektive metod hade verkat. Testet med ultraljud utfördes en gång, medan testet med AOT utfördes två gånger. Ultraljud visade sig vara bäst på att reducera biofilmstillväxt; med en reducering på 68 till 100 %. Oberoende av material minskade ultraljud tillväxten på samtliga plattor. AOT visade tvetydiga resultat; från det första testet tycktes det påverka produktionen av biofilm, medan den inte visade lika tydliga resultat i det andra testet.

Acknowledgement

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Anna Hjalmars
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Abbreviations and explanations

AMBIO	Advanced Nanostructured Surfaces for the control of biofouling
AOP	Advanced Oxidation Processes
AOT	Advanced Oxidation Technology
CIP	Cleaning in place
CMS	Cotswold Microsystems LTD
CNT	Carbon Nanotube
DLC	Diamond Like Carbon
DNA	DeoxyriboNucleic Acid
DTI	Danish Technological Institute
EN	Electroless Nickel
EPS	Extracellular Polymeric Substances
GPS	Gross National Product
LP	Low Pressure
MIC	Microbial Influenced Corrosion
MICI	Microbial Influenced Corrosion Inhibition
NT	New Technology
ORP	Oxidation and Reduction Potential
PDMS	Polydimethylsiloxane
PECVD	Plasma-Enhanced Chemical Vapor Deposition
PFTE	Polytetrafluoroethylene
PHE	Plate Heat Exchangers
PS	Polystyrene
SRB	Sulfur Reduction Bacteria
SS	Stainless Steel
TBT	Tributylin
TNO	Netherlands Organisation for Applied Scientific Research
UV	Ultraviolet

1. Introduction

Many industries encounter problems with fouling. Fouling can be a severe problem as it affects the performance of the equipment and accounts for additional maintenance costs in form of money and time.

Biofouling is a phenomenon that occurs in environments where the concentration of bacteria is high and where there are available surfaces for them to grow. This is well-known in the marine industry; for centuries boat hulls have been victims of bacterial growth and it has been solved in different ways; for the past century mostly by the usage of paints containing biocides. As many of these biocides are highly eco toxic, the usage of some has been banned internationally. The most known example is probably Tributylin (TBT), that was banned in 2003 due to its bio toxicity, bioaccumulation and widespread effects on organisms in the aquatic environment [1-2].

Besides boat hulls, biofouling is a problem in other industries and applications, such as cooling towers and heat exchangers. For cooling towers, this is often solved by adding biocides to the system, whereas for heat exchanger maintenance is done by mechanical cleaning or insertion of a Cleaning In Place (CIP)- unit.

1.2 Aim

The purpose of this thesis was to study the fouling phenomena, and more specifically biofouling on Plate Heat Exchangers (PHE). Additionally, the purpose was to identify and evaluate different methods to reduce fouling and especially biofouling, both commercially available and methods found on the research level. Two methods were then selected in order to evaluate their practical performance.

1.3 Limitations

The selected methods should be environmentally friendly- in such that they should not contain any hazardous compounds-, and they should be possible to use them in PHE applications. The chosen methods could either be coatings that can be applied onto the surface, or physical methods that treats the water used in the heat exchanger.

Part I: Theoretical "Biofouling in heat exchangers"

2. Background

2.1 Fouling

Fouling is often used as a generic name for different processes that result in foulants on materials. It occurs when one material is in constant contact with a fluid. It can be precipitation of solids dissolved in the fluid onto the surface - sometimes referred to as scaling-, sedimentation of particulates, chemical reactions between the fluid and the surface material, biological growth, or a combination of these. Sometimes corrosion is also classified as fouling. Fouling is caused by several different factors such as the chemical and physical properties of the fluid and of the surface material, as well as the temperature and velocity of the fluid [3].

Chemical properties of interest are: the fluid itself, pH, alkalinity, oxidation and reduction potential (ORP), the salt concentration, compounds and particles dissolved in the fluid. The higher the concentration of dissolved salts, the higher the risk of fouling. For most salts, an increase of temperature results in an increase in solubility. However, for some compounds the relation is the opposite; the higher the temperature the lower the solubility. Example of these salts are $CaCO_3$, $Ca(OH)_2$ and $Mg(OH)_2$. These compounds are called inverted soluble compounds [4-6].

pH affects the structure of some compounds, microorganisms and materials. It also affects the solubility of some compounds such as CO_2, which in turn affects the solubility of $CaCO_3$ [4].

It is important to know the velocity of the fluid, since low velocity increases the sedimentation rate both for particulate matter and biological organisms. Figure 2-1 shows how different types of fouling in heat exchanger are dependent on the velocity [4].

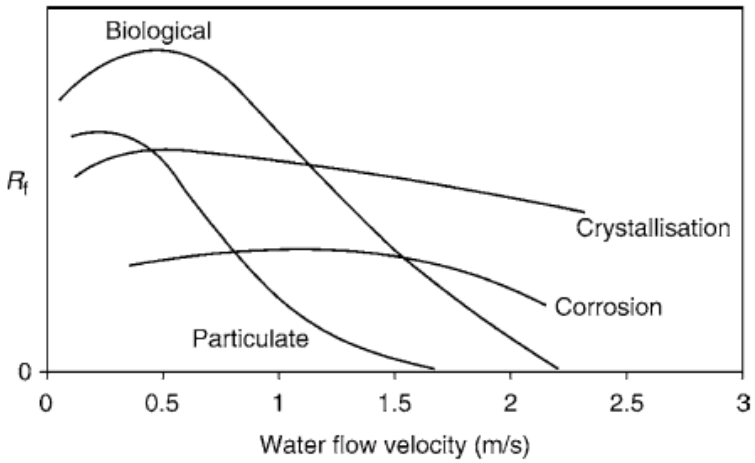

Figure 2-1: Velocity dependency for different types of fouling in heat exchangers. The higher the Rf-value, the more fouling. [7] (with permission)

Another problem concerning the velocity is turbulent backflow. Turbulent backflow occurs when the viscosity is low, the velocity is high and the pressure changes excessive. Backflow increases the temperature in the region, and consequently the fouling rate of inverted soluble compounds and other types of fouling [8-10].

The temperature is not only interesting concerning solubility; it is also important for microbial growth and the chemical reactions within the bulk and on the surface. The optimal temperature for biological growth ranges from 15^0C to 50^0C, the exact value differs between different bacterial species. An increase in temperature in the bulk can either increase or decrease reactions in the bulk, depending on the compounds within it. High temperatures on the surface can cause degradation of compounds in the fluid, resulting in a coke on the surface. Sometimes this process can even cause polymerization resulting in shorter oligomers with a rubberlike appearance. Figure 2-2 shows the surface temperature dependency for some fouling mechanisms [4] [8].

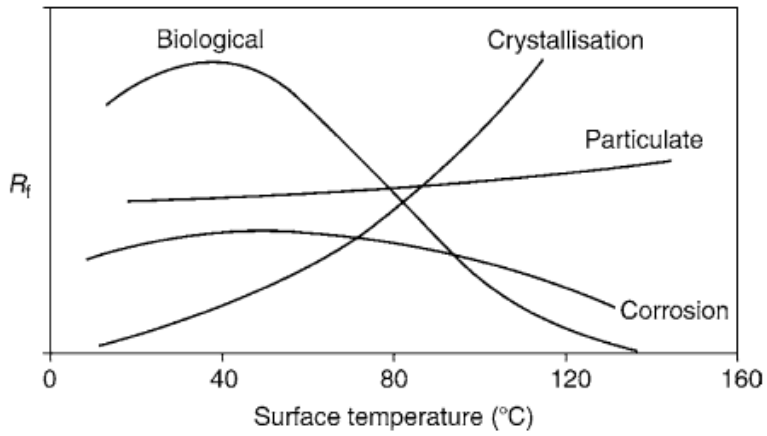

Figure 2-2: Temperature dependency for different types of fouling in heat exchangers. The higher the Rf-value, the more fouling. [11] (with permission)

Concerning fouling, the surface material is highly essential. Some materials are charged in neutral pH, which makes attachment of compounds easier. The physical properties of the material, as the morphology and scratches on the material are also important. Scratches are sites for fouling build-up, as particulates and molecules can sediment into these and induce further fouling.

2.1.1. Stages in fouling

Fouling starts with an induction period, which is the time before a notable fouling starts. This period can range from a few minutes to several months depending on the different parameters. Low surface temperature, low surface roughness and high flow rate increase the induction time. After the induction period the fouling rate follows a constant pattern that can either be linear, falling, saw tooth or asymptotic. Most industrial processes show a asymptotic behavior, *i.e.* it reaches a steady state [12-15].

2.2. Biofilm

For microorganisms it is favorable to be part of a biofilm, as it provides nutrients and protection. For example, bacteria within a biofilm are more resistant to antibacterial agents. More than 90 % of the bacteria in the biosphere live in biofilms. Natural biofilms are heterogeneous, *i.e.* there are several different species of bacteria within the biofilm. The bacteria are arranged in the best organized way, in order to draw advantages from each other. In order to communicate most microorganisms use signal substances. This process is called Quorum sensing and is used to regulate different processes within the biofilm and in the microorganism itself. It is also used as an indication to planktonic bacteria to know the number of bacteria already situated in the biofilm. Known signal substances are oligo-

peptides and acylated homoserinelactones. Most fouling bacteria use acylated homoserinelactones [16-18].

2.2.1 Formation of biofilm

Biofouling can either occur by itself or in a combination with other fouling processes. If the concentration of bacteria in a fluid is high it is likely for biofilm formation to occur. The beginning of biofilm production highly depends on the properties of the surface material. Nevertheless, as soon as a biofilm is produced, the continuous process of biofilm production is independent of the surface. The first step starts with a buildup of an electric double layer between the fluid and the surface. This electric double layer either enhances or reduces (depending on if the layer is attractive or repelling) the attachment of organic compounds such as proteins and polysaccharides to the surface. These attached molecules form a film, called the conditioning film. Besides electrostatic charges, this process is governed by the surface energy and hydrophobicity. Due to diffusion rate and Brownian motion, *i.e.* random motion of small particles, small molecules settle first. By time these molecules are exchanged to larger molecules as they might have better affinity to the surface [19-20].

The second step in the building of the film is attachment of bacteria. This process proceeds by Brownian motion, sedimentation and convective mass transport. The forces between the conditioning film and the bacteria are mainly interactive forces, such as Van der Waals, hydrogen bonding etc. [20-21].

Bacteria that attach to a surface change their phenotype from planktonic, *i.e.* free swimming, to bacteria in a biofilm. When this occurs the flagella on the bacteria disappears and the bacteria starts to produce extracellular polymeric substances (EPS). The EPS contains proteins, carbohydrates, DeoxyriboNucleic Acid (DNA), uronic acid and water. Between 75 % to 90 % of the biofilm is made of EPS. Gram negative bacteria produce anionic or neutral EPS, while Gram positive bacteria also may form cationic substances. The EPS reinforces the attachment, often referred to as irreversible attachment. Even though irreversible attachment is taking place, microorganisms in the biofilm can mitigate and establish new sites with biofilms. After a while larger organisms attach to the surface, as larvae and barnacles. This fouling process is called macrobiofouling [19] [20-23].

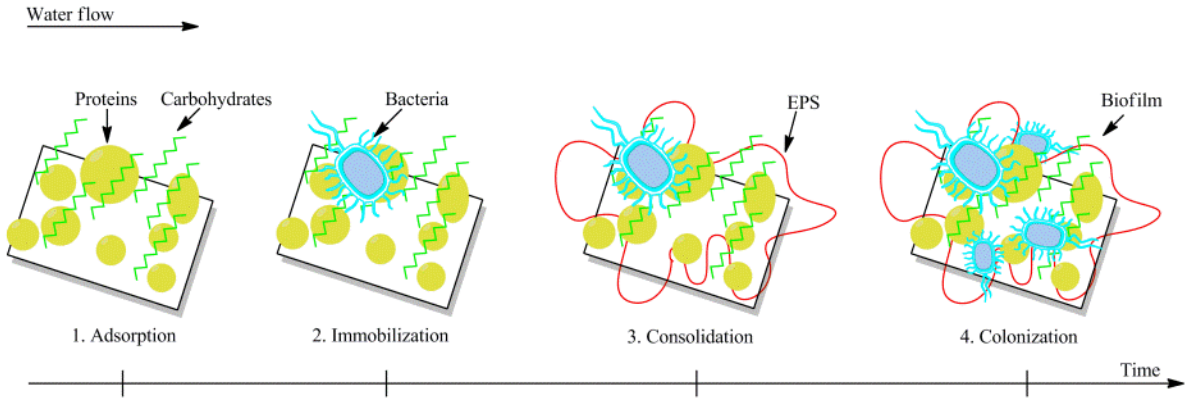

Figure 2-3: The attachment process of biofilms. 1) Proteins and carbohydrates attach to the surface forming the conditioning film. 2) Bacteria attach to the surface 3) Bacteria produce EPS which reinforces the attachment. 4) Biofilm formation is completed and bacteria from the film are released and find new places for attachment, also higher organisms can attach to the biofilm during this process.

Conditions for biofouling to occur

Some conditions need to be fulfilled for biofouling to occur. For example, the temperature needs to be in a certain range; higher than 120^0 C no bacterial growths occur. The optimal temperature is around

15 ^{0}C to 50 ^{0}C depending on the bacteria, see figure 2-2. A maximum for bacterial growth was found to be at 35 ^{0}C [4]. Nutrients, such as phosphor, nitrogen and trace elements must be available. For aerobic bacteria the oxygen level in the water needs to be above 4 mg/L. In completely stagnant water biological growth is unlikely due to oxygen and nutrients deficit. However, if the velocity is too high biofilm gets dragged off. 1.5 to 2 m/s seems to be a sufficiently high velocity to drag off biofilm. Optimal velocity for biofilm formation is about 0.5 m/s, see figure 2-1. The velocity also affects the density of the biofilm; higher velocities induce formation of denser biofilm up to a certain level [24-27].

Biofilm detachment

Biofilm detachment is the most limiting factor regarding biofilm thickness. Detachment can be caused by physical and/or chemical changes. Examples of physical changes are abrasion and erosion. Chemical changes can be oxygen deficit, decrease in EPS production, among other reasons. It can also be caused by external factors such as grazing of eukaryotic cells or conditions causing starvation in the biofilm [28-29].

Combination effects

Bacteria and surfaces are often negatively charged and this induces a repellant electrical layer. If a cation is present and attaches to the surface, the surface is neutralized and the repellant electrical layer reduces. This phenomenon promotes the attachment of negatively charged molecules and bacteria to the surface. The thickness of the electrical double layer is inversely proportional to the square root of the ionic strength. Thus, higher ionic strength means thinner electrical layer, *i.e.* weaker force. Hence, a high concentration of cations present in a solution can increase the rate of biofouling, by their reduction of repellant electric layer. According to earlier studies this is true for some bacteria, however, not for all [30-31].

Other studies have shown that different cations have different effects on bacterial attachment that could be either increasing adhesion ability or decreasing depending on the cation and bacteria [31]. Some studies believe that electrostatic layer has a minor effect on the attachment of the bacteria itself, as the attachment of bacteria occurs mainly through the conditioning film [20] .

Cations also seem to influence the production of EPS for some bacteria; some work have shown reduction of Ca^{2+} and Mg^{2+} concentration decreased the production of carbohydrates [31].

Microbial influenced corrosion (MIC) and Microbial influenced corrosion inhibition (MICI)

Microorganisms on metallic surfaces can either enhance or undermine cathodic or anodic reactions. The process is influenced by several factors, such as the EPS from the bacteria, the chemistry of the surface, temperature, pressure and pH. An example of a mechanism is sulfur reduction bacteria (SRB) present in some biofilms that can remove hydrogen from the surface and consequently an increased oxidation of the surface [32-33].

2.3. Industrial equipment

Fouling occurs in all systems in contact with water or other fluids containing high concentration of substances. Industries using cooling water are prone to fouling and biofouling. Biofilm can form on waste water systems, storage tanks, pipes and cooling water systems. For some industrial equipment, the fouling problem is more severe than others. Heat exchangers using sea and lake-water are often susceptible to biofouling [24] [34].

2.3.1. Plate Heat Exchangers (PHE)

There are several different types of heat exchangers and they can be classified according to different criteria such as construction, heat transfer mechanism, recuperation or regenerators, transfer process and flow arrangement [35].

Plate Heat Exchanger is the second most common type of heat exchanger after shell heat exchanger. The advantages with PHE are the high heat transfer coefficient (U), the compactness and the large surface area density that is between 120-230 m^2/m^3. The disadvantages with PHE are that they have a narrower temperature and pressure span compared to tube and shell heat exchangers. An example of a heat exchanger is seen in Figure 2-4 [36].

The material used in heat exchangers depends on the properties of the fluids and the working temperatures; it can be different types of stainless steel (SS), brass, copper, titanium and nickel. The most common material in Sweden for heat exchangers is 316 L stainless steel. Nevertheless, for heat exchanger in contact with seawater or brackish water titanium is more often used, due to its resistance to corrosion [24] [34] [37-40].

PHE:s consist of several corrugated or flat plates that either are welded, brazed or conjunct together with gaskets. The plates are corrugated to obtain turbulent flow. The distances between the plates are around 1.3 to 2 mm. For corrugated plates the smallest distance between the plates can be even shorter. The flow of the cold fluid and the hot fluid flows on different sides of the plates, see Figure 2-4. The acceptable flow in the heat exchanger varies and depends on the application. The flow is limited by the pressure drop and geometrical design. Many PHE have an upper limit of 5000 m^3/h. Generally the flow is about 0.3 to 0.9m/s for water like fluids, however, the velocity can be up to 4 times higher in some regions depending on the construction. For PHE made of SS the recommended velocity is below 10 m/s. Above this velocity the erosion risk is too high. For titanium the value is higher as it has better resistance to erosion [39-43].

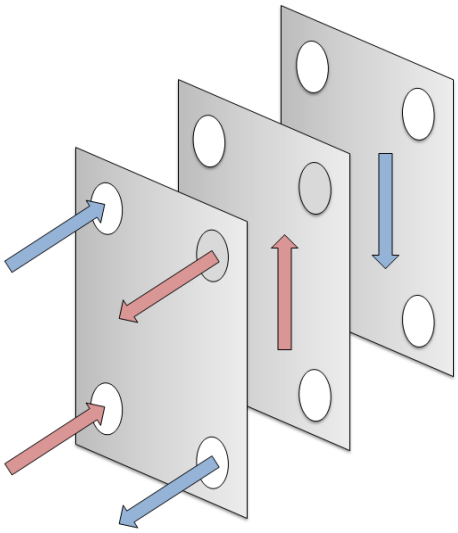

Figure 2-4: Principle of a flat PHE. The hot and the cold stream flow on each side of the plate. On the one side of the plate the hot fluid travels upwards and on the other side of the plate the cold fluid downwards.

Fouling in plate heat exchangers

Fouling in heat exchangers can be caused by several different factors, mentioned in section 2.1. Due to the risk of fouling the size of a heat exchanger is often overestimated by 30-50%. Several types of combined fouling mechanisms occur in a heat exchanger process. The critical problem with fouling on heat exchangers is the reduction of heat transfer and increase of pressure drop. The cost of fouling on heat exchangers is estimated to be 0.25 % of Gross National Product (GNP) in industrialized countries [24] [44-45].

Generally, fouling on PHE is less than for tube and shell heat exchanger. This is due to two facts; the uniform and the dominant turbulent velocity profile inside PHEs. The uniform profile eliminates zones of low velocity. The turbulence decreases the settlement and increase the removal of deposits. A disadvantage with PHE is the compact structure, which increases the fouling rate and also has a more severe impact on the heat transfer compared to tube and shell heat exchangers [24] [44].

As mentioned above, the velocity profile in PHE is generally turbulent. Nonetheless, close to the surface the flow is laminar and fouling generally occurs in the laminar region. Several reports have shown that a decrease in laminar sublayer results in a decrease in fouling, suggestively due to increased shear stress [46]. Tests have shown that heat exchanger working with fluids with high tendency of fouling should have a wall shear stress over 50 Pa to eliminate fouling [14] [26] [47].

The decrease in heat transfer can be calculated by using a fouling resistance factor. This factor is empirically found and more commonly used for tubular and shell heat exchangers than plate. The equation describing the heat transfer coefficient with the fouling resistance considered is as follows [14] [44] [48].

$$\frac{1}{k} = \left(\frac{1}{\alpha_1} + R_{f,1}\right)\frac{A_2}{A_1} + R_{wall} + \frac{1}{\alpha_2} + R_{f,2}$$

α, A and R_f are the heat transfer coefficients, heat transfer areas and the fouling resistances, respectively. Index 1 and 2 represent the two different fluids. R_{wall} is the thermal resistance of the separating wall and k is the actual heat transfer coefficient. Some R_f-values are represented in Table 1 [4].

Table 1: Some resistance factor values for different types of cooling water used in plate and frame heat exchanger applications. [4]

Water specification	**R_f [m^2K/kW]**
Distillated water	0.009
River water	0.043
Coastal seawater	0.043

Close to the heat transfer surface the temperature lay in the range for good biofilm growth. Cooling water systems usually operate at a temperature between 20^0C and 50^0C, which is in the range of optimal growth, see figure 2-2. A biofilm has a thermal conductivity of 0.6 W/m,K and a 250-micron thick layer of slime may result in a 50 % reduction of heat transfer [16] [49-50].

Describing fouling on heat exchanger

Usually fouling begins with an induction period, which is sometimes considered when modeling the design equation. Some models have been made to describe fouling in PHE. These models were one, two or three dimensional. Three dimensional descriptions are more interesting when a detailed picture of a certain area is to be studied [51].

Particulate and crystal fouling are usually divided into several processes; firstly the reaction within the fluid, secondly the mass transport of the foulant, thirdly the formation and attachment of the deposit on the surface, fourthly the release of the deposit and last aging of the deposit. Describing fouling is problematic as many factors affect the result. From lab modeling result it is difficult to scale up to larger systems. For example, in small systems reaction in the bulk is one of the limiting factors for deposition on the surface, whereas in larger systems the mass transport to the surface is the limiting factor [52].

2.4 Water qualities

The water quality is of importance concerning fouling; the water provides the foulants and the microorganisms to the surface. For heat exchangers common used waters are sea and fresh water. As sea water is more abundant and the usage of fresh water is more restricted; seawater usage is more common than fresh water. Nowadays, the medium to be cooled is generally in a closed system, *i.e.* the coolant water is in indirect contact with the medium and after usage returned to its origin [34].

The water can be used in three different ways; either by direct or indirect contact with the hot medium, or through cooling towers. For cooling systems using sea water, corrosion and biofouling are the most common types of fouling [34] [53].

2.4.1 Fresh water

The type of fouling that is dominant in fresh water highly depends on the composition of the water. Factors that influence the type of bacteria are chemical composition of the water, temperature, pH and oxygen level [24].

Cooling water systems using fresh water often have problems with *Pseudomonas fluorescens* bacteria [25].

Eastern Mälaren

Lake Mälaren is the third largest lake in Sweden, with a surface of 1000 km^2. Eastern Mälaren has moderately high amounts of phosphor and nitrogen. During late summer and autumn large areas suffer from oxygen deficit, meaning that during these periods only anaerobic bacteria can survive. The bacterial growth in the surface water (0-4 m) has declined during the past 40 years from around 500 bacteria/100 ml to less than 100 bacteria/100 ml [54-55].

The canal of Karlberg

The canal of Karlberg is a small canal situated between Kungsholmen, Vasastaden and Solna, in Stockholm, Sweden, see Figure 2-5. It contains high amounts of phosphor and high numbers of bacteria from May to September. Normally the number of bacteria is over 100 bacteria/ 100 ml, however, it is often as high as 1000/100 ml [56].

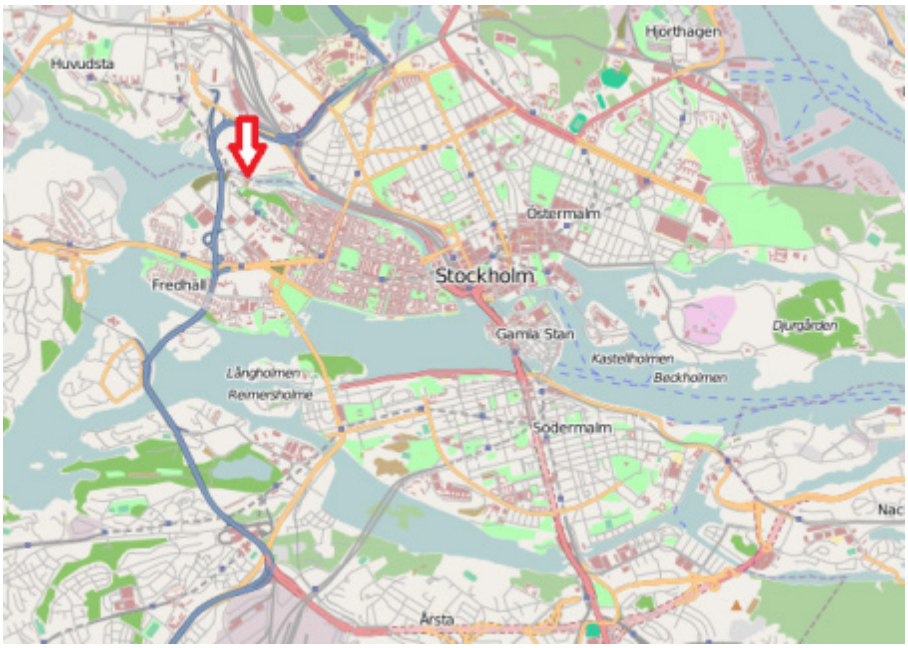

Figure 2-5: The arrow points out Karlbergskanalen. [57]

2.5 Materials and attachment ability

The used material is of great importance concerning biofouling. Generally low surface energy means low cell attachment. Other important parameters are topography and hydrophobicity of the surface, pH, temperature as well as the characteristics of the present bacteria. The crucial characteristics are charge, hydrophobicity and appendages of the bacteria. As the hydrophobicity of bacteria partly depends upon external factors, these characteristics can change and hence the affinity to the material [58-61].

Surface energy and hydrophobicity do not have a straight linear relationship, however, for many cases it is assumed to have a linear relationship, by applying Youngs's equation see below [62-64].

$$Y_s = Y_l \cos \Theta + Y_{sl}$$

Where Y_s is the surface energy for the solid, Y_l is the surface energy for the liquid and Y_{sl} is the interfacial energy between the solid and the liquid. If $\Theta > 50^0$ the surface is assumed to be hydrophobic, if $\Theta < 50^0$ it is assumed to be hydrophilic.

Protein, polysaccharide and cell attachment is low on hydrophilic surfaces when the surface-water interfacial energy is low [58] [65].

2.5.1 Polystyrene

Polystyrene (PS) is an artificial polymer that has a hydrophobic surface, as can be seen from its structure, see figure 2-6. The surface energy ranges from 30 to 43 mJ/m^2. Some studies have shown that bacteria show a relatively low adhesion to PS, *as L. monocytogenes* and *Staphylococcus aureus* [59-60]. Other papers claim the opposite, *i.e.* that *L.monocytogenes* and also *Salmonell,* have a high adhesion to hydrophobic material such as PS [61] [66]. The different results from these studies can be attributed to the fact that the adhesion mostly depends upon the current characteristics of the bacteria.

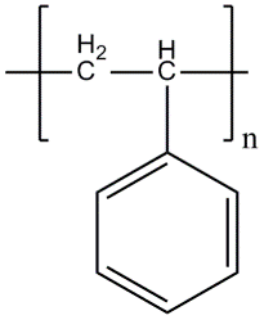

Figure 2-6: Structure of polystyrene.

2.5.2 Titanium

Titanium has an excellent resistance against corrosion, and is therefore often used when the coolant is seawater or brackish. It can also be used in a large temperature range, up to 300^0 C, and at applications where high velocities are needed. On the other hand, titanium is often exposed to biological growth. When titanium is in contact with air a small thin oxide layer (about 5 mm thick) forms on the surface. This oxide layer becomes a protective layer that inhibits further damage to the material. Depending on the structure and composition of the oxide layer the surface can either be hydrophilic or hydrophobic [34] [24] [67-70].

A study comparing biofilm production on SS and titanium in water used in a nuclear plant, showed that the biofilm production was more than three times faster on titanium than on SS. Besides the rate of biofilm growth the bacterial flora in the biofilm differed. *Ralstonia* and *Mycobacterium* was most common in the biofilm on titanium whereas *Bacillus* and *Stenotrophomonas* where more common in the biofilm formed on SS [71]. Another test performed on coastal seawater showed similar results; that more biofilm was formed on titanium than on SS [50].

2.5.3 Stainless Steel

For freshwater systems the most common material for heat exchangers is stainless steel. SS is an alloy used for its anticorrosion properties and its good temperature resistance. Most studies have proven SS to be hydrophilic and due to this SS has problems with biofouling when the surface-water interfacial energy is high. SS shows a high adhesion to some bacteria, e.g. *Staphylococcus aureus* and *L. monocytogenes,* in comparison to PS [24] [60] [66] [72-73].

316 L Stainless steel

316 L is the most commonly used material in heat exchangers in Sweden, as previously written. It has a surface energy about 40 mJ/m^2. The composition of 316 L stainless steel is shown in Table 2 [37] [72].

Table 2: Composition of 316 L stainless steel. [74] [75]

Element	Composition [%]
Carbon	Max 0.03
Manganese	Max 2
Phosphorus	Max 0.045
Sulfur	Max 0.03
Silicon	Max 0.75
Chromium	16-18
Nickel	10-14
Molybdenum	2-3
Nitrogen	Max 0.10
Iron	Balance

2.6 Solutions

Different types of solutions exist in order to remove fouling. It can either be mechanical cleaning *in situ* or temporary. It can also be modifications of the surface or application of a surface coating. Traditionally, coatings have been used for boat hulls in order to inhibit fouling. In heat transfer equipment, coatings are seldom used due to practical difficulties in maintenance, and the reduced heat transfer coefficient. When fouling has reached a crucial stage in plate heat exchangers, cleaning is mainly accomplished with CIP-liquid or mechanically. For many years CIP-liquid has contained chlorine. Producers of PHEs recommend to do back flushing once a week, and performing mechanical cleaning every 2 to 10 years depending on the water quality. Preventive solutions are oversizing the heat exchanger, settling tanks, filter or strainers adjacent to the heat exchanger [37] [76-79].

2.6.1 Commercially available

Coatings

Coatings applied to reduce fouling can be divided into two groups depending on their approach to reduce fouling. Either it can be an "antifouling coating" or a "fouling release coating". Both of these coatings have traditionally been used on boat hulls. Traditional antifouling coatings contain biocide, which can be copper, zinc pyrithione or other similar compounds [80-83].

There are different approaches to decrease fouling in coatings; by increasing or decreasing the hydrophobicity or by decreasing the surface energy and/or the surface morphology. The coatings can either use one of these techniques or a combination of them. Hydrophilic coatings often contain silicone. The principle of these coatings is based on softly adherence of the bacteria that releases under a certain shear stress. In order to be used in heat exchanger applications, these coatings should preferably be thinner than 1 μm, so that they do not affect the heat transfer performance [80] [84].

Silicone based coatings

Coatings based on polymers of either pure SiO_X or combined with CH- chains have showed antifouling properties, and some versions of these coatings are available on the market or under development.

Sol gel process

The sol gel process is a method developed 50 years ago, where liquid together with glass ceramics cure and forms a flexible coating through polymerization. The curing temperature ranges from 140^0C

to 200^{0}C. Several companies are developing different polymer coating based on sol gel process to achieve antifouling properties [85-86].

Organosilicon compounds created through a sol gel process

The Danish Technological Institute (DTI) has developed a sol gel coating, CORE Coat 010, for heat exchangers that has been tested on Alfa Lavals products. The sol gel reduces the surface free energy and the roughness. The coating can be applied to a final thickness of 5 μm and the surface energy is 20 mN/m^2 [87].

Physical technologies

Ultrasound

Ultrasound is a technology that has been used for several years to clean devices in industry. For heat transfer applications the first studies begun in the 60s, however the deeper studies only begun in the 90s. One of the first patents for ultrasound on heat exchanger to control biofouling is from 1981 [88]. The past decade several patents are made for ultrasound in heat exchanger applications. However, most of these patents are for tube and shell heat exchangers [89].

The principle is based on physical and kinetic changes in the medium. The ultrasound is produced via a transducer. When exposed to a positive signal the transducer expands, when exposed to a negative signal the transducer contracts. This motion induces waves in the medium, and if these are above a frequency 18 kHz the sound is ultrasonic. The two most important cleaning features of ultrasound is the kinetic and physical change in the water. The kinetic change increases the mass transport and decreases the diffusion layer, which result in an increase of the dissolution rate and the mass transport from the surface to the bulk. The physical change is the disruption of the biofilm structure by the waves [90-93].

However, the most effective process is cavitation, *i.e.* tiny bubbles that are formed from the compression and decompression of the water, see figure 2-7 below. The compression is called positive pressure and thus the section of no compression is called negative pressure. If the negative pressure is sufficiently low (below the pressure for vapor formation) small cavities are formed. Cavities can either be stable or instable; meaning that they explode. Stable cavities occur when the intensity is sufficiently low. When instable cavities explode in the end of the compression cycle, the cavity produces heat and pressure and small microjets. The heat and the pressure may generate formation of free radicals, as $OH\cdot$, and compounds such as H_2O_2. Formation of cavities depends upon several factors, such as the tensile strength of the liquid, *i.e.* the force needed to separate two molecules, the hydrostatic pressure, the specific heat of the liquid and the type of gas in the bubble, the temperature and the frequency from the transducer. The frequency must be lower than 2.5 MHz in order for cavitation to occur. Ultrasound at high frequencies, on the other hand, can induce deformation and cause damage in the cell walls of bacteria allowing water to enter. The penetration of water into the organism contributes to expansion of the cell leading to its death. Important factors regarding the effectiveness are the frequency and the intensity of the ultrasound and exposure time. The amplitude of the wave has also been mentioned as an important factor [91-92][94-97].

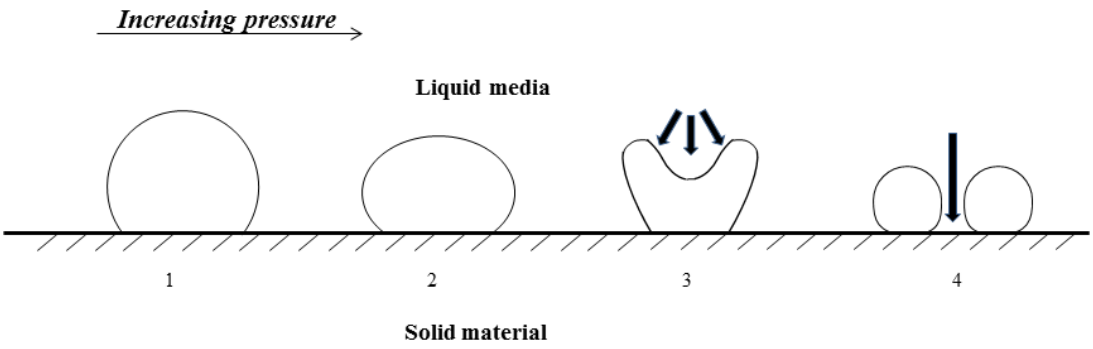

Figure 2-7: Formation of cavities and later microjet from a solid surface.

Ultrasound and bacteria

Several studies of bacteria and ultrasound have been made with different results. Some reports have shown that low frequency (frequencies <700 kHz) and high intensity (larger than 5 W/cm^2) have a high biocidal effect [93], whereas other has shown that higher frequency (about 800 kHz) results in a better biocidal effect [98]. Research on biofilm on implants have shown that a frequency of 500 kHz and intensities of 20 mW/cm^2 and 50 mW/cm^2 have a small biocidal effect [99]. Studies with low intensity (<2 W/cm^2) and low frequencies proved an enhancement on the bacterial growth, due to increased nutrient, waste transport and low cavity formations [100]. The precise frequency and intensity depends on the bacteria to be removed and the form of the biofilm. Some bacteria show a resistance to ultrasound, for example *Pseudomonas aeruginosa*, which might be related to the effects of ultrasound on the quorum sensing mechanism [94].

Several research studies have shown that best detachment performances for biofilms are obtained at a frequency between 40-100 kHz and high intensities over 10 W/cm^2. Tests with lower intensity than 10mW/cm^2 have shown no biofilm disruption [94] [101].

A test where ultrasound has been applied in intervals, 30·3 seconds ten times per 24 hours, with a frequency of 20 kHz and moderately high intensity shows a complete control of biofilm production in tubes with 88 % reduction compared to the control sample. During the same study a test to remove already existing biofilm was performed. The result proves that biofilm could be controlled when the distance from the tube and the transducer was less than 50 cm [25] [79].

However, many studies claim that ultrasonic treatment has little practical potential, as it is not effective for all microorganisms and spores. According to these studies ultrasound should be used in combination with other techniques, such as heat or pH change [97].

Ultrasound for other types of fouling and applications

Tests when ultrasound has been used as a cleaning tool for boat hulls have shown good results. The tests were performed at 20 kHz and an intensity of 300 W/cm^2. The transducers were installed outside the hull where it moved back and forth. The transducer was used after the hull was fouled. The results were good when the distance from the transducer to the surface is sufficiently short, around 2 mm [102].

Tests with ultrasound on microstructured heat exchanger fouled with calcium carbonate show a relative good performance when the frequency was 20 KHz, amplitude of 1 mm, intensity of 900 mW/cm^2 and the ultrasonic waves were applied in pulses. The ultrasound was placed directly on the surface of the heat exchanger. According to the authors there was no cavitation as the intensity was low. Unfortunately, the results did not show complete removal of the deposits [103].

Advanced Oxidizing Technology

Advanced Oxidizing Processes (AOP) are based upon generation of highly reactive species, such as $OH^{\cdot}$, $O_3^{\cdot}$, O_3 and H_2O_2. Advanced Oxidizing Technology (AOT) is a development of AOP. The formation of the radicals can be accomplished through different process, such as UV-light, ozone, cavitation etc. The combination of UV-light and a semiconductor at the surface of the apparatus is an efficient way degrading several compounds. UV-light and additionally hydroxyl radicals created from UV-light absorbed at the semiconductor degrade compounds. A commonly used semiconductor is titaniumdioxide [104-106].

Semiconductor

The concept with a semiconductor is based on the interplay with UV-light and the conductor. In the semiconductor crystals the molecular orbitals are combined and form a broad valence band. When a crystal is illuminated by a light with sufficiently high energy excitation of electrons occur. After excitation has occurred, a free electron and a positive electron hole are formed. The electron can either return to the initial condition or it can induce reduction of compounds and species near the surface of the particle. Likewise, the electron hole can induce oxidation. These oxidation and reduction reactions can form radicals. It is believed that the most commonly produced radical is the hydroxyl radical. Hydroxyl radicals can then further react and induce other reactions [107].

UV-light and bacteria

The mechanism of disinfection is based on absorption of ultraviolet (UV)-light by proteins, RNA and DNA in the bacteria. The dosage of light needs to be 10 times the fluence, H_0, for the bacteria. H_0 is the dosage in J/m^2 that affects a bacteria, and this number differs depending on the specie. Biological effects are achieved from a UV-light of 190-350 nm. The nucleotide bases have their absorption maxima around 260 nm. The mechanism depends on a change in the chemical structure of the nucleotide. For example, when two thymine bases are situated close to each other and absorb UV-light, a thymine dimer can be produced. This change makes it impossible for the organism to replicate and thus disinfection is achieved. Some bacteria manage to repair these types of damages, called dark- or photo- reactivation, example of these organisms are *Pseudomonas aeruginosa, Salmonella typhi* and *Salmonella typhimurium* among others [108].

Bacterial inactivation through a semiconductor

When a hydroxyl radical is produced either the hydroxyl radical itself, or other radicals produced from chain reactions, can react with the cell membrane of the cell. In gram negative bacteria the first target is lipopolysaccharides, whereas in gram positive the peptidoglycan layer and proteins are the first targets. The reactions can cause further reactions to occur on the membrane and inside the cell. The produced chain radicals can reach as far as the nucleus. Theories exists that peptidoglycan layers inside the cell membrane may act as a protection for further reactions. However, this theory has not clearly been proven yet [109-110].

Low pressure mercury arc lamp

In UV-disinfection low pressure (LP) mercury lamp is extensively used. The output is generally about 30 to 50 W. They provide monochromatic light at 253.7 nm and 184.9 nm when synthetic quartz of high purity is used [111].

AOT and biofilm formation

Few academic studies have been made on AOT and biofilm formation. According to a case study, where an AOT was installed in a cooling tower system, AOT had an effect on the growth. However, no statistical tests were accomplished to confirm that there was a significant difference [112].
Several studies have been made on UV-lights impact on biofilm formation alone. The results from the tests are ambiguous, some show that UV-light has an effect on formation, others not. In combination with other techniques/biocides such as H_2O_2, UV-light has a better impact on biofilm [113-114].

Other physical methods

Some power plants have tried to reduce biofouling by testing different physical techniques. Sudden changes in the flow direction, and the flow velocity were performed. Both of these techniques washed away accumulations of fouling organisms. Thermal shock from rapid cooling or heating has also shown a good performance. On the other hand, this might lead to clogging of loose organisms in the system [50].

Design modifications

New Technology (NT)-plates

New Technology (NT)-plates are plates that are designed differently from ordinary plates. By increasing the width of the channels furthest away from the incoming stream, see figure 2-4, the velocity profile over the plate is more uniform. In this way zones of laminar flow are eliminated which leads to a decrease in fouling [115].

Solutions used in other applications

Cleaning balls

Taprogge GmbH developed in the early fifties a solution for fouling, where elastic cleaning balls are used to clean the system from fouling. The balls are elastic and sized just above the tube size (normally it exceeds the tube size with 1-3 mm). When the ball enters, the ball expands and by this it removes the fouling on the walls. The balls are injected through a strainer and then recollected into a collector and this is accomplished through pressure differences. This process occurs at certain intervals and is performed continuously. Depending on the application the balls vary in hardness, diameter and cleaning frequency. For titanium- and stainless steel- tubes the average cleaning degree is 12 balls/hour. The lifetime of the ball is about 4 weeks. More than 12, 000 units from Taprogge have been installed. Even though several units have been installed, the technique has been criticized for being too expensive and ineffective on extensive fouling. Another drawback is that it has only been used for tube and shell heat exchangers [16] [116].

Sonic Horns

Since the 90s several companies have been selling acoustic horns supposed to be used for cleaning applications in industrial scale. These horns are made of a diaphragm and a horn. The principle is that pressure differences around the diagram flex it and by this produces a sound at around 200 Hz. The sound travels through the horn to the surface where it is supposed to clean. These horns are specialized on particulate cleaning on tube and shell heat exchangers and are in general used in gas systems [16] [117-118].

Figure 2-8: A picture of a sonic horn. [119]

Good results have been shown for loose deposits. For sticky deposits the horns have not shown as good results. The drawbacks with the technique is the sound pollution, as the used frequency lies in the range of human hearing, as well as the possible damage the vibration can cause to the material [16].

2.6.2. Research

Coatings

Carbon nanotube-polytetrafluoroethylene

Carbon nanotubes (CNT) in a matrix of hydrophobic polytetrafluoroethylene (PTFE) have been tested on a plate heat exchanger used in a diary process in laboratory scale. The result showed 70.3% less fouling than the uncoated surface.

An observed problem was that CNT attach weakly to metal surfaces, thus CNT can easily be peeled off. This can have crucial consequences as CNT potentially have toxic effects [120].

Sepiolite clay in a sol gel process

Netherlands Organisation for Applied Scientific Research (TNO) is developing Nanohybrid sol-gel coatings incorporating clay nanofillers that supposedly can be used for heat exchanger applications. The matrix is made of sepiolite clay with polysiloxane incorporated. Currently this coating can be less than 50 μm thick. In laboratory the coating has good thermal properties, and antifouling properties. However, in field tests the coating did not show good antifouling properties on heat exchangers. Tests with different amount of polysiloxane incorporation show a better fouling release property, however, not a significant reduction of bacteria adhesion [80] [121].

Diamond like carbon (DLC)

DLC has good thermal conductivity; similar to metals, corrosion resistance, hardness, wear resistance, and a smooth surface, which makes it a good candidate as a coating. It has been used for several

different applications, from medical devices to tools. The surface energy of DLC is around 45-50 mJ/m^2. DLC can be attached by plasma-enhanced chemical vapor deposition (PECVD), via a silicon carbide matrix that enhances the attachment. A disadvantage with DLC is that it attaches poorly to stainless steel [49] [122-126].

Tests with DLC have shown a 35 % removal of attached bacteria. Due to the amorphous structure of DLC other elements can be incorporated into the coating. Studies have shown that incorporation of silicon, nitrogen or fluorine has lower surface energy and antifouling properties than plain DLC. [124] Combination of elements may show an even better antifouling property. DLC with incorporated Si showed a 78 % less bacterial attachment compared with plain 316L stainless steel [123]. Plain DLC showed a 20% less biofilm adhesion than an uncoated material, and 50 % less for DLC incorporated with PTFE [127].

Several producers of DLC coatings exist on the market; however, all of these coatings are purposed for hard high wear materials. Teer Coating Ltd has during the Advanced Nanostructured Surfaces for the control of biofouling (AMBIO) project developed DLC incorporated with Si and N proposed to be used for heat exchangers. Nonetheless, for the moment they are not commercially available [80] [126].

Silicon oxide-like coatings deposited by vapor deposition

Teer Coating has developed a coating based on a $[(CH3)3Si]_2O$ monomer. The coating is applied on the surface through PECVD. The exact content of the final product is not known. Previous studies when parameters as ion cleaning time, deposition time, and amount of silicone vs. CH were varied, showed that the best antifouling performance was achieved with a high amount of hydrocarbon and a long ion cleaning time. Ion cleaning is accomplished prior to deposition of the coating to achieve a clean surface. Longer ion cleaning time increases deposition rate and hence the crosslinking of the polymer coating. The achieved film ranges a thickness from 400 nm to 1200 nm and the surface energy ranges between 20 to 23 mJ/m^2 [80] [128].

3. Selection of methodology for reduction of biofilm

To select the appropriate methods, some criteria must be fulfilled. The methods must be commercially available and the methods should preferably have been tested on PHE applications or similar. The methods should also be environmentally friendly, in such that they do not produce or emit harmful compounds. As the availability of commercial coatings is limited, it was decided to try two physical methods; Wallenius Waters AOT product and ultrasound.

Part II: Experimental

"Biofouling on SS, titanium, PS and AOT and ultrasound impact on biofilm"

4.1. Purpose

The purpose of the study was to evaluate two different methods to decrease biofouling on different materials. The chosen materials were stainless steel, titanium and PS submerged in water from lake Mälaren. The tested methods were AOT and ultrasound.

4.2 Choice of material and test set up

Stainless steel and titanium were chosen as they are the two most common materials in PHEs. 254 SMO stainless steel plates were used since these were already present in the lab, and the chemical composition of 316 L and 254 SMO is similar; see appendix A for composition of 254 SMO and table 2 for composition of 316 L. PS is a commonly used surface regarding biofilm formation, and was for that reason also used.

The water used in the test was taken from the lake Mälaren, as it is a good model for water used in heat exchanger applications in Sweden and for its accessibility from the lab.

A plastic tank was used in the AOT test due to its appropriate size for the experiments. For the ultrasound test it was decided to use a small steel bucket, as it was believed that the waves would travel better through a steel medium than through plastic.

4.2. Limitations

The staining method is a semi- relative quantitative method, showing the amount of produced biofilm relatively to other results. Consequently, the analysis does not tell the absolute amount of produced biofilm, neither the type of bacteria within the biofilm.

The results do not tell the precise affinity of bacteria to different materials, as the biofilm forming bacteria on the plates were not analyzed. The results are only a general indication of the affinity for the materials in the type of conditions tested in the scope of this study.

The quality of the water before the experiments started was only analyzed through dip-slides and temperature measurements. No analysis on the type of bacteria, neither the quality of the water such as; pH, nitrogen or phosphor analysis were performed prior or during the experiments. These parameters can affect the number of bacteria and accordingly the biofilm formation. It was assumed that they did not vary throughout the test trails.

4.3 Experimental set up

4.3.1 Advanced Oxidation Technology

Test: Biofilm production of different materials and the impact of AOT

Material

Table 3: Materials used for the experimental set up.

Description	Provider
Freshwater	Mälaren, taken from Klarabergsviadukten
Plastic tank, 70 liter	-
Polystyrene plates	Plastintime AB
254 SMO Stainless steel plates [1]	Stockholms Vattenskärning
Titanium plates	-
0.25 kW Pump	BUSCK
0.7 kW pump	GVIP
AOT 5-05	Wallenius Water

Analysis

Table 4: Chemicals used for the analysis.

Description	Provider
Crystal Violet CAS no. 42555	MERCK
Denatured ethanol 95 %	VWR Chemicals
Ethanol 95 %	Kemetyl
TTC dip-slides (1.00778 Cult Dip Combi)	MERCK
Nutrient agar	Nutrient agar - 9 cm Karolinska institutet

Arrangement

Two tests were performed. The second test was a replicate of the first test with some modifications.

In the first test, the plates were placed in a 70 liters tank with recirculation. Water from lake Mälaren was taken two to three days prior to the experiments. The tank was filled with 62.5 liters of water. The plates were submerged in the water at specific positions in the tank, showed in table 5. The plate that was closest to the edge of the tank and the one furthest away were situated 2 cm and 11 cm from the edge respectively.

[1] See appendix A for composition of 254 SMO Stainless steel

Figure 4-1: Arrangement. The plates are hanging from wooden crosses, and are placed in the order showed in table 5 and table 6.

Table 5: Placement of the plates in the first test.

Placement from the inflow (clockwise) [^{0}]	Number of plates	Material	Distance between plates [cm]	Distance from water level [cm]
120	6	Stainless steel	1.5	42
210	6	Polystyrene	1.5	42
300	6	Titanium	1.5	42

The three inner plates (closest to the edge) from each position were removed after the first 10.5 days and then analyzed. At the same time the tank water was replaced with new water from Mälaren and three new plates of each material (titanium, PS and SS) were added to the system and an AOT apparatus was installed. All the 18 remaining plates were analyzed after 10 additional days.

For the second test, the order of the plates was changed so that the plates of different materials were rearranged around the tank. The order of the plates is shown in table 6.

Table 6: Placement of the plates in the second test.

Placement from the inflow clockwise) [^{0}]	Material (no. 1 is closes to the edge)	Distance between plates [cm]
60	1. Stainless steel	1.5
	2. Polystyrene	
	3. Titanium	
	4. Polystyrene	
	5. Titanium	
	6. Stainless steel	
210	1. Titanium	1.5
	2. Stainless steel	
	3. Polystyrene	
	4. Stainless steel	
	5. Polystyrene	
	6. Titanium	
300	1. Polystyrene	1.5
	2. Titanium	
	3. Stainless steel	
	4.Titanium	
	5. Stainless steel	
	6.Polystyrene	

The same procedure was performed as for the first test with the only difference being the order of analysis. After the first 10 days, the three outer plates from position 60^0 and 300^0 and the three inner plates from 210^0 were analyzed, instead of only analyzing the inner plates. These plates were replaced with new ones of the same materials, the tank was filled with new water, and an AOT was installed.

4.3.2 Ultrasound

Test: Biofilm production of different materials and the impact of ultrasound

Material

Table 7: Materials used for the test set up.

Description	**Provider**	
Freshwater	Mälaren, taken from Klarabergsviadukten	
Stainless steel tank (12 liter)	Flinks järn	
Polystyrene plates	Plastintime AB	
254 SMO Stainless steel plates	Stockholms Vattenskärning	
Titanium plates	-	
Aquarium pump	-	
Ultrasound device	Sonic Shield II provided by Cotswold Microsystems LTD (CMS) Marine	Specifications
		20-40 kHz
		Pulses every 500 ms
		50 W
		3,14W/cm^2

Table 8: Chemicals used for the analysis.

Description	**Provider**
Crystal Violet CAS no. 42555	MERCK
Denatured ethanol 95 %	VWR Chemicals
Ethanol 95 %	Kemetyl
TTC dip-slides (1.00778 Cult Dip Combi)	MERCK
Nutrient agar	Nutrient agar - 9 cm Karolinska institutet

Arrangement

The test was performed in two sections the first part was a reference test without the ultrasound device and the second part was the actual test with the ultrasound device installed.

A small stainless steel bucket tank was filled with 9 liters from Mälaren. The water was taken in plastic buckets two and one day prior to the experiment respectively. Nine plates of different materials were placed in the tank: three of SS, three of PS and three titanium plates. They were placed in groups of three in the middle of the tank with a distance of 6 cm, from each other and 3 cm from the water surface. In order to obtain oxygen to the water, a small aquarium pump was attached. The bucket was covered with aluminum foil to minimize evaporation. After 10 days the plates were analyzed with a staining method, see section 4.4.

For the second part of the test (when ultrasound was operating), the same arrangement was used with the only difference that one transducer was placed under the bucket and the plates were placed slightly deeper under de surface (4.5 cm instead of 3 cm). See figure 4-2 for arrangement.

Figure 4-2: Arrangement for the ultrasound tests. On the left the ultrasound device can be seen attached to the bottom of the tank. On the right the plates and the tube from the aquarium pump can be seen.

4.4 Analytical methods

In order to detect formed biofilm a staining method was used. The principle of the method is that both active cells and extracellular matrices are stained with the color Crystal Violet. Crystal Violet is a basic dye that stains both negatively charged extracellular molecules and cellular surfaces. Ethanol is a semi-polar solvent that enables dissolving of the dyed biofilm. Crystal Violet in an ethanol solution absorbs light from 450 nm to 650 nm and has its maximum at 590 nm [129-130].

The method has been used in previous studies. However, the procedure used in this study is slightly modified from these. First the plates were stained with a 0.1% Crystal Violet solution for 45 minutes, thereafter dipped in distillated water, and finally dried. For the first AOT experiment the plates were dipped twice in distillated water and for the second AOT experiment the plates were dipped nine times. For the ultrasound tests the plates were dipped four times. The film was then desorbed with 95 % ethanol, until all the color disappeared. At the end, the absorption of the ethanol solution was analyzed in a spectrophotometer at 540 nm [131].

During the experiments, dip slide analyses were performed. Dip slide is a common and cheap method to measure bacterial growth in water systems. A dip slide is a small plastic device covered with nutrient agar. The slides were incubated at 30^0C or 37^0C for minimum 48 hours and then the number of bacteria is counted. This gives an indication of the number of bacteria in the water. The method has a measurement error of factor 10 [132].

During the second AOT test and during the ultrasound experiments, the surfaces of the plates were swiped with swabs. Afterwards the swabs were swiped onto nutrient agar plates and incubated at 37 respective 22 ^{0}C for one week. This was fulfilled in order to be able to verify if there actually was any bacterial activity on the plates.

4.5 Results

4.5.1 Results from the tests: Biofilm production of different materials and the impact of AOT

The number of bacteria varied from 10^3-10^5 bacteria/ml during the test periods. There were from 10-100 times more bacteria during the first AOT experiment than during the second.

There was a bacterial activity on all the plates according to the agar plates. However, the activity was low and slow at 22^0C. For the plates that were incubated at 37^0C there was a higher activity, see appendix C.

Figure 4-3 to figure 4-8 show the absorbance of the plates in absorbance/cm^2 plate. The first number under the bars is the time the plates have been in the tank, in days. The second number is the order of the plates from the tank edge, where 1 means closest to the edge. AOT means the plates that only have been in the water when the AOT was operating. The exact placement in the tank can be seen in table 5 for the first test and table 6 for the second test.

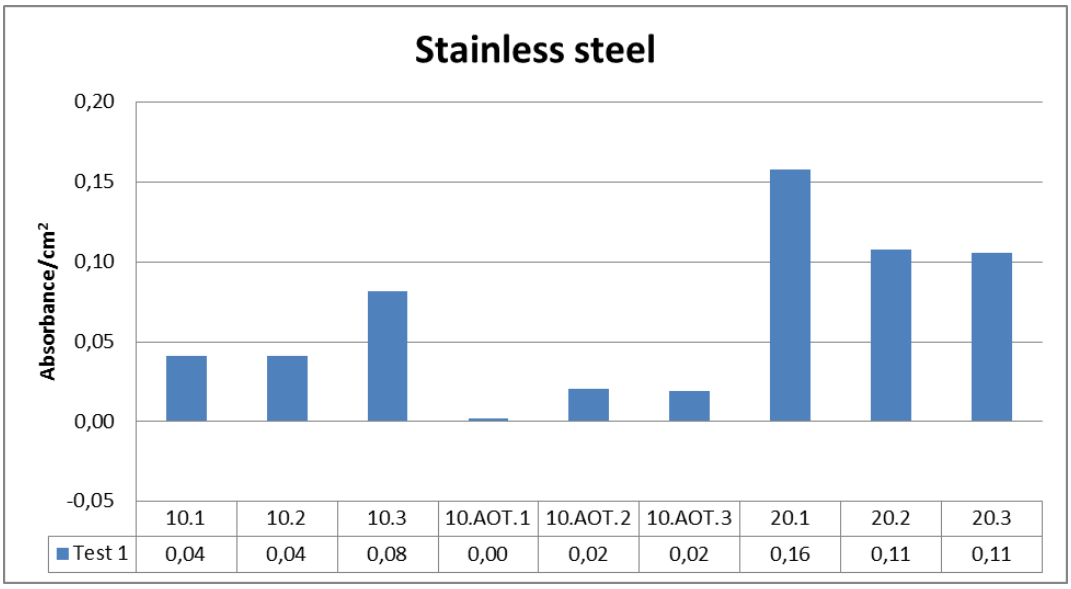

Figure 4-3: Results of the absorbance from the SS from the first test.

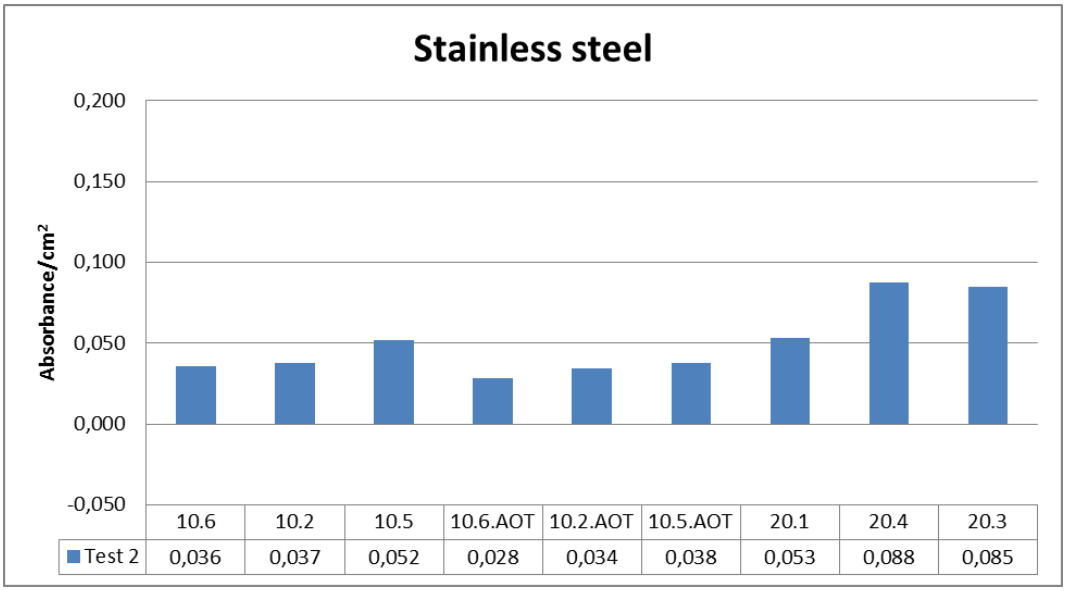

Figure 4-4: Results of the absorbance from the SS from the second test.

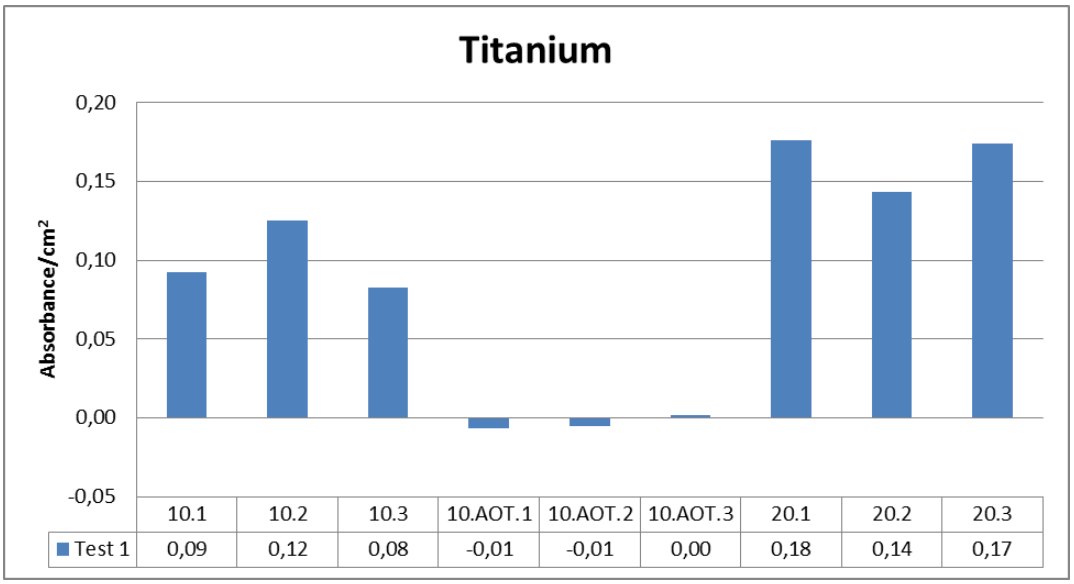

Figure 4-5: Results of the absorbance from the titanium plates from the first test.

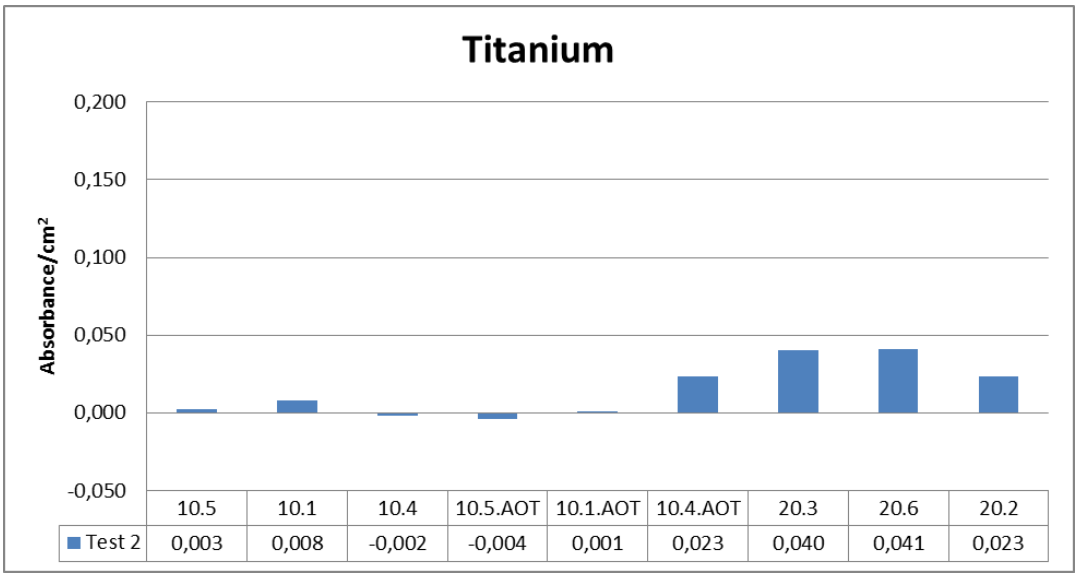

Figure 4-6: Results of the absorbance from the titanium plates from the second test.

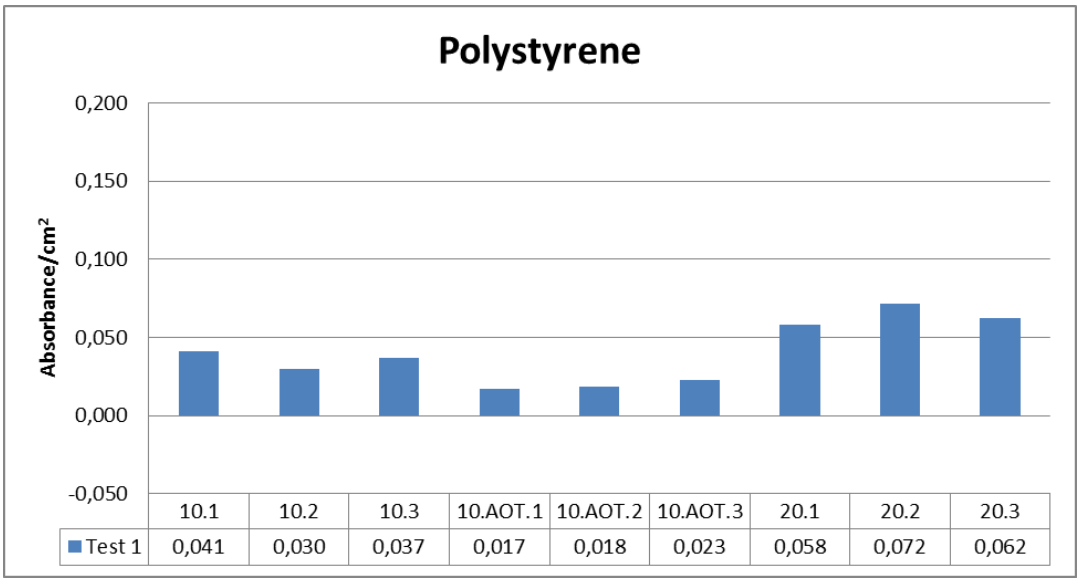

Figure 4-7: Results of the absorbance from the PS plates from the first test.

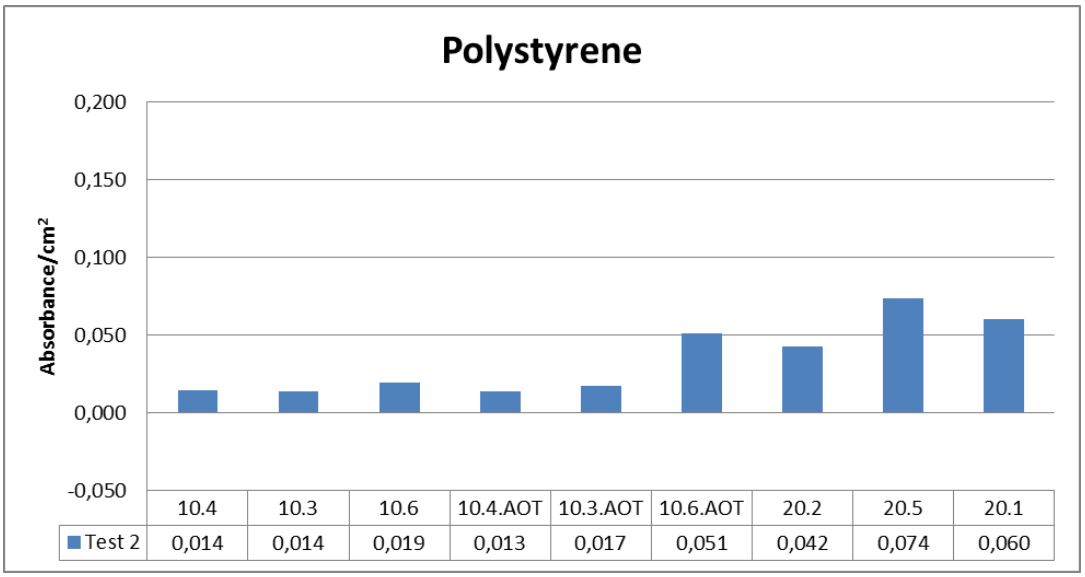

Figure 4-8: Results of the absorbance from the PS plates from the second test.

Table 9: The decrease in produced biofilm with AOT vs. without and the average increase of biofilm during the last 10 days.

Test trail	**Material**	**Decrease/Increase of biofilm. The result without AOT compared with the result from AOT.**	**Average increase of biofilm the 10 last days.**
1	Titanium	-98%	260 %
	Stainless steel	-75%	100 %
	Polystyrene	-46%	130%
2	Titanium	-	1000 %
	Stainless steel	-20 %	240 %
	Polystyrene including all numbers	74 %	170 %
	Polystyrene without the extreme large number	-2 %	280 %

4.5.2 Results from the test: Biofilm production on different materials and the impact of ultrasound

Two dip slides were taken; one during the reference test and the other one when the ultrasound had been operating for 3 days. Both slides showed a bacteria number of less than 10^3.

When ultrasound was not operating the production of biofilm is fairly high, as can be seen in figure 4-9 to figure 4-11. When ultrasound was operating, the formation of biofilm was considerably lower, which also can be seen in figure 4-9 to figure 4-11. The results from the agar plates support the results from the Crystal Violet analysis; see appendix C for photographs taken of the agar plates.

Figures 4-9 to figure 4-11 show the biofilm production on titanium, SS and PS plates. The first three bars are from the plates submerged in the water when the ultrasound was not operating. The three last ones are from when ultrasound was operating. The first number represents the position in the bucket and the second number the order within the group. The US (ultrasound) suffix identifies the plates submerged in the tank during ultrasound operation. From the results it can be seen that there is on average 100%, 68% and 78% less biofilm on the titanium, SS and PS plates respectively when ultrasound was operating compared to the reference plates.

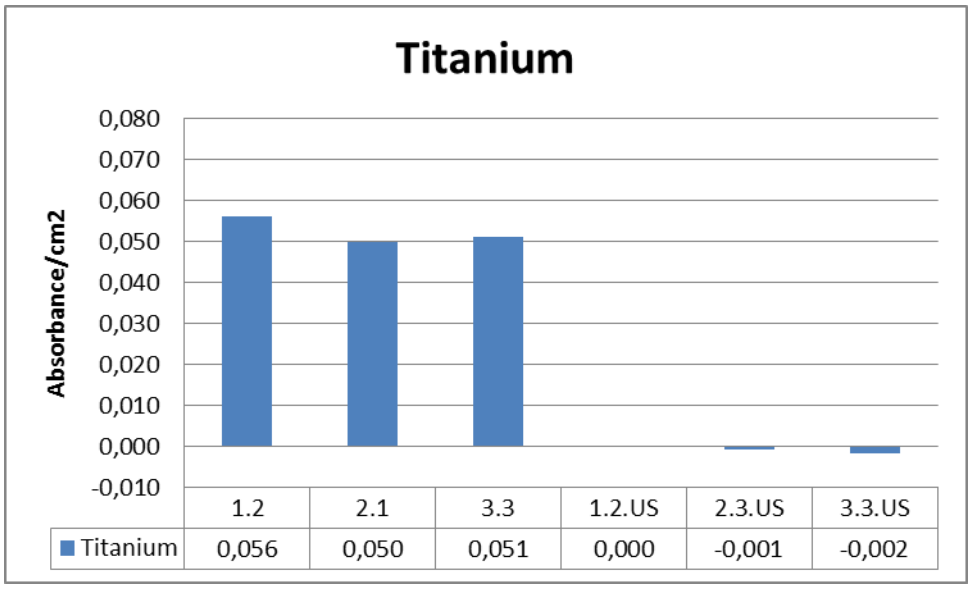

Figure 4-9: Results from biofilm production on titanium plates.

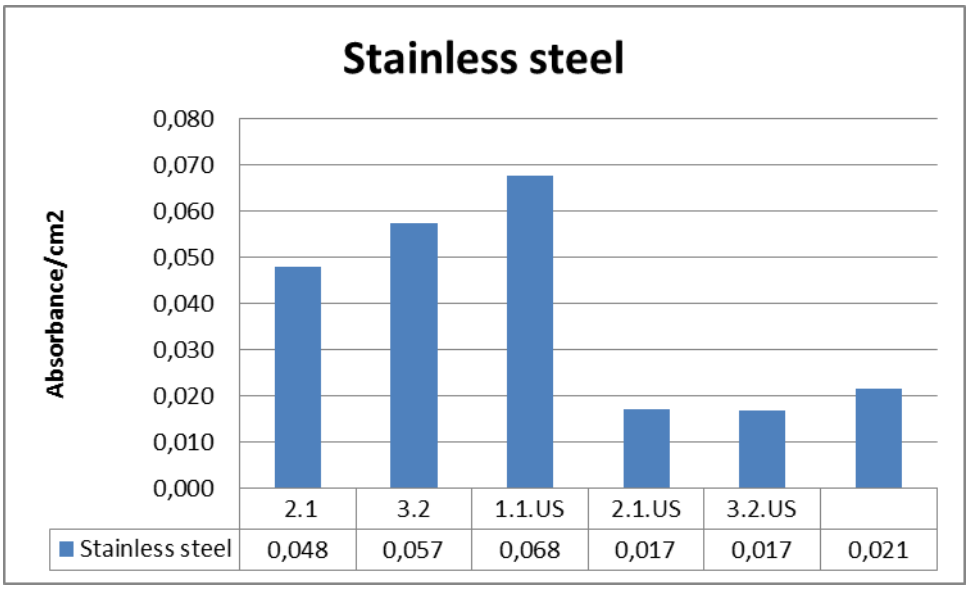

Figure 4-10: Results from biofilm production on SS plates.

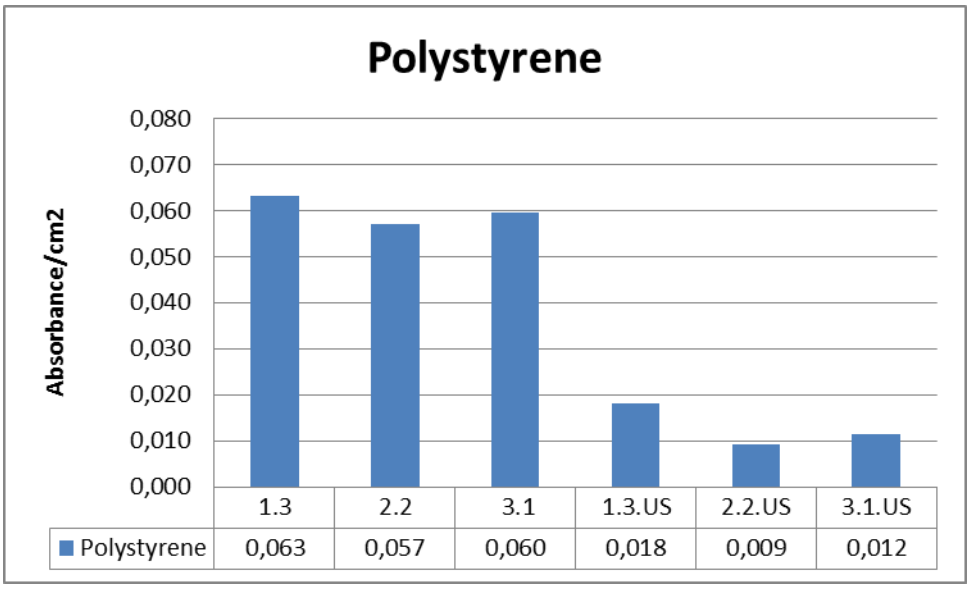

Figure 4-11: Results from biofilm production on PS plates.

4.6 Discussion

4.6.1 Biofilm formation on different materials

In general, the dip slides showed higher number of bacteria in the water than what *"Vattenprogram för Stockholm"* claimed that the canal of Karlberg has, see section "The canal of Karlberg" [56]. According to *"Vattenprogram för Stockholm",* the number of bacteria is around 10 bacteria/ml [54]. The big difference might be attributed to three reasons; firstly, the water used in this study was placed in a small plastic bucket in room temperature for 1-3 days. Secondly, the water samples were also taken from the shoreline. Lastly, it could be the possible measurement error of the dipslide. Inside the plastic bucket probably a biofilm and bacteria already existed, as well as the growth of bacteria is for most bacterial species faster at room temperature than at 10 degrees. Water from the shoreline has presumably higher concentration of bacteria than for the canal of Karlberg in general. As the measurement error of dip slides is about a factor 10 this could mean that the bacterial growth was actually lower than what the dip slide showed.

There was also a significant difference in the production of biofilm during the second test between titanium and PS, as well as titanium and SS for all the time periods except for the plates that were in the tank for 10 days with AOT, see appendix D for statistical data. During the first experiment, biofilm production was highest on the titanium plates. In the second experiment there was hardly any biofilm on the titanium plates the first 10 days, and only a little after 20 days. As can be seen from figure 4-5 and figure 4-6, there was actually a negative biofilm production, which means that the results are not reliable. However, the result from the agar plates showed that bacteria existed on the plates (see appendix C for pictures of the agar plates) meaning that there was a bacterial growth on these. Nevertheless, the growth was not sufficiently high to be detected or too little and therefore easily removed by shear force.

The different results between the titanium plates from the first and the second experiment can be attributed to either a measurement error or a difference in the active bacteria in the water from the first test to the second test. The incoming water temperature was lower in the second experiment, as well as a slight difference in the number of bacteria, from 10^4 - 10^5 bacteria/ml in the first test and 10^3bacteria/ml in the second, see appendix E. According to section 2.4, different bacteria attach to

different types of materials. The lower temperature might reduce the type of bacteria that attach better on titanium. The pictures taken of the agar plates for the plates during the ultrasound test show differences in colonies (see appendix C); on the titanium plates there are only yellowish colonies, whereas there are white colonies on the PS. The reason for this might be that the bacteria forming white transparent colonies are hydrophobic and have less affinity to titanium material. Interpreting the colonies on agar plates is difficult as many bacteria show the same appearance on agar plates [133]. Therefore, species are not studied during these tests, thus, no real conclusions can be drawn regarding the characteristics and affinity of the bacteria. The yellowish colonies could be colonies of *Chryseobacterium daecheongense*, as they form yellow marks on nutrient agar and are found in freshwater [134]. The white colonies could be bacteria from the *Pseudomonas* genus as they often produce white colonies on nutrient agar [135]. However, defining bacteria is complex and difficult; therefore, no conclusions can be drawn on the actual species in the biofilms [136].

According to the studies made by Montero *et al*. [71] and K.K. Satpathy *et al*. [50], biofilm was produced faster on titanium than on SS. This would be true for the first AOT test, however, not for the second and neither for the ultrasound experiment. Nevertheless, the results from those studies might be true for their scope of study, however, not in general, and therefore the results from this study differ from theirs.

There is a general trend that more biofilm is produced on SS than on PS. The difference is significant for two periods; the plates that were in the tank for 20 days during the first test, and the plates that were in the tank for 10 days without AOT in the second test, see appendix D for statistical data and numbers. However, these results are not in accordance with results from previous studies where biofilm of *Staphylococcus aureus* and *L. monocytogenes* grew faster on PS than SS [60] [73]. Additionally, the results are not consistent as it was the opposite relation for the plates in ultrasound reference test, as well as for the plates in the first test with AOT that were immersed for 10 days, *i.e.* more biofilm on the PS plates than the titanium. Nonetheless, the differences in the two latter cases are so small and can therefore be neglected.

4.6.2 Impact of AOT on formation of biofilm

Previous studies have shown inconsistent results of UV-light and its effect on biofilm formation. UV-light in combination with H_2O_2 has shown a better biofilm control. Lakretz *et al*. claim that this occured due to the formed hydroxyl radicals. Hydroxyl radicals are also produced in AOT 5-05, presumably it should influence biofilm formation. This was supported for the first test, nevertheless not for the second [114].

According to the results from figure 4-3 to figure 4-8, AOT had an impact on biofilm formation. For the first experiment, the formation of biofilm during AOT- operation was 50-98% less than when AOT was not operating. All these results were significant according to statistical testing, see appendix D. For the second experiment the results are fairly different: they proved only 2-20 % less biofilm production, when extremely high value from PS is disregarded (0.051 absorbance/cm^2, see figure 4-8). If this value is considered, there is an increase of production of 74%. None of these results are statistically significant.

The large difference between the two tests could be attributed to the dipping and the temperature. Generally during the second test there is less detected biofilm than during the first test, except for SS under AOT operation where more biofilm was detected on the second than the first. The reason for this could either be measurement error, or that there was actually more biofilm during the second experiment.

The difference in results could also be attributed to the total number of bacteria in the water. In the second experiment there were 10-100 times fewer bacteria in the water. Fewer bacteria mean reduced ability to produce biofilm, which in turn mean less biofilm on the plates. With little biofilm on the plates changes between a reference and another test are more problematic to observe. Thus, the impact of AOT in the second test might be more difficult to observe, due to the lower number of bacteria in the water.

4.6.3 Impact of AOT on already existing biofilm

For all the plates that were in the tank for 20 days there was an increase of biofilm, meaning that AOT does not have an impact on existing biofilm. The results do not tell whether AOT influences the rate of development, *i.e.* if the formation of biofilm might have been larger if the AOT had not been operating.

4.6.4 Impact of ultrasound on biofilm development

Seen from figure 4-9 to figure 4-11, ultrasound has a clear impact on biofilm formation. This is also confirmed by the agar plates. To confirm the reliability of the results, the test should be performed at least one more time.

In this study, a low frequency and a low intensity ultrasound device was used. According to previous studies, ultrasound waves under these conditions should enhance the biological growth. However, in the previous study the experiments were performed under shorter periods of time (1-48 hours of ultrasound exposure) and at a slightly higher frequency (70 kHz) and slightly lower intensity (2 W/cm^2) [100]. According to the theory, exposure time is an important factor concerning bacteria detachment. This could be the reason for the better bacterial detachment compared to the study made by Pitt.*et al*. Another reason could also be the lower frequency during these tests.

There cannot be a clear statement whether cavities were formed during this experiment or not, as formation of cavities depends upon several factors. However, higher power and accordingly higher intensity is needed to form cavities. Therefore, most probably there was no cavitation under this experiment. The loss of biofilm is probably attributed to the increased shear forces at the surface and a shorter diffusion layer which makes adhesion more difficult [92-93] [97].

In general, ultrasound had the largest influence on biofilm on titanium. The reason for this is probably the loose attachment of bacteria to the material, which increases the efficiency of the ultrasound. Presumably, ultrasound has a better effect on the whiter colonies than on the yellowish, as they were absent during the ultrasound test. In figure C-3 in appendix C, in the picture on the left upper corner the white colonies can be seen. The reason for the different results for the white and the yellow colonies could be attributed to hydrophobicity; the white colonies could presumably be more hydrophobic than the yellow ones. This is supported by previous studies, which claim that ultrasound has different impact on different colonies [92].When ultrasound was not operating the biofilm on titanium might have consisted of the bacteria giving the white colonies on the agar plates. When ultrasound was under operation these colonies could have been removed from the surface, as they presumably were loosely attached to the surface.

4.7 Source of errors

General

For the first AOT experiment the temperature of the water was 10^0 C resp. 11 ^{0} C when arriving to the laboratory. Before the test started the water temperature was at room temperature, *i.e.* 19^0 C. During the test the water was 27^0 C when the AOT was not in use. When the AOT was operating the water

temperature was 31^0 C. For the second experiment the water had a temperature of 8 and 7^0 C respectively when arriving to the laboratory. An immersion heater was also added after 3 days of operation to obtain the same temperature as when the AOT was used. For the ultrasound test the temperature of the water was 5 and 8^0 C respectively when arriving to the laboratory. Before and during the experiment the water temperature was at room temperature, *i.e.* 19^0 C. As bacterial growth is highly temperature dependent all these facts should have influenced the results.

The shape of the titanium plates and the steel plates were different from the PS ones. The PS plates were square and had smooth edges, whereas the steel and titanium plates were rounded with rough edges. At the edges, crystal violet can attach and result in a higher absorbance. The PS plates were also covered with a protective plastic film (attached with electrical forces) that was removed just before the plates were placed in the tank. The titanium and SS plates were not covered with a plastic film. Therefore, there could be more scratches on these plates enhancing either biofilm production or crystal violet attachment during staining. Both of these facts are to some extent taken into consideration as the absorbance of a blank is removed from all the results.

When the plates in the second AOT-experiment and the ultrasound experiment were swept with swabs, the plates were not dried prior to sweeping. This could mean that the grown bacteria colonies on the agar plates came from the water and not from the biofilm as desired.

Generally dipping is a vital part of the analytical method that affects the results. Dipping can either be performed fast; resulting in a large shear force, or it can be performed slowly resulting in a longer time of immersion in the distillated water. Both of these should affect loose biofilm. Biofilm that is mature or developed might not be affected in the same way as immature biofilm. According to reports, cells that are not enmeshed in the biofilm and daughter cells are more susceptible to shear stress [29] [137]. Immature biofilms probably have higher number of daughter cells and cells that are not enmeshed around the surface, as the growth rate is faster. Hence, immature biofilm should be more affected by shear stress. In all the tests small amounts of biofilm were detected, which makes it assumable to consider them as immature biofilms. It is therefore presumable that dipping has a big impact on the results. For this reason, a test was accomplished to determine the impact of dipping. The test supports that the number of times of dipping influence the results to a certain extent, as well as the measurement error. At least there is a difference in not dipping at all and dipping more than 4 times, see appendix F for these test results.

4.7.1 AOT test

Some of the plates touched each other, or did not stand perfectly stable in the tank, which might have resulted in less production of biofilm.

In order to use less material, the PS plates had been cut in half for the second experiment; this results in non-smooth edges, which might lead to increased production of biofilm or Crystal violet attachment.

For the first test with AOT, the plates were only dipped twice, which resulted in tiny drops of crystal violet solutions on the surface of the plates. This would lead to a higher absorbance than the real value. As all the plates were dipped twice, as well as the blank, it is assumed that the amount of drops would be around the same for all the plates. Thus, the relative results should be roughly correct. To eliminate the possible measurement error in drop formation, it was decided to dip the plates 9 times for the second test.

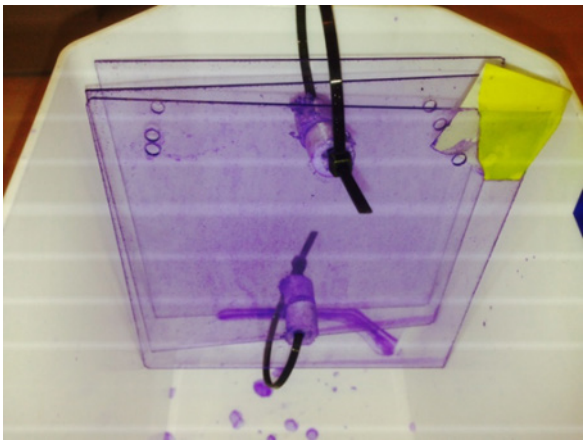

Figure 4-12: The plates after being stained and dipped twice.

The produced biofilm was in general scarce, which means that the measurement errors have a large impact on the results. For example, the titanium plates from the first test period of 10 days with AOT, and all the titanium plates that were in the tank for 10 days from the second test, showed negative biofilm production. When subtracting this negative amount from the results on the other plates, the value reaches almost zero, *i.e.* the measurement error is almost as big as the detected biofilm. Consequently, none of the results from the titanium plates from the second AOT test are reliable.

The plates that were in the tank 20 days were placed into a small box when the water was exchanged. This might have resulted in a small loss in biofilm.

Under the second test, the pump stopped operating sometime during a weekend. As a consequence, the water was not recirculating for 0 to 2 days. As this occurred when AOT was installed, AOT could not treat the water during this time period, *i.e.* the concentration of bacteria most likely increased during this time period.

4.7.2 Ultrasound test

The tube from the aquarium pump was not static during the test, which resulted in that sometimes it was not immersed in the water. This led to less oxygen in the water, which might have had an impact on the results.

During the second part of the test the plates were immersed deeper in the water than during the reference test. It might mean that the plates during the reference test had access to more oxygen meaning that the bacteria could have grown faster. As the size of the bucket was small (12 L), the distance for diffusion was short, *i.e.* oxygen concentration should have been about the same within the bucket. Thus, the level of immersion probably had a minor effect.

5. Conclusions

There is a slightly higher production of biofilm on stainless steel compared to polystyrene in all the tests, probably due to the types of bacteria in the water taken from Mälaren.

AOT probably decrease the biofilm formation. However, the results are not significant.

AOT does not have an impact on existing biofilm, meaning that biofilm continue to grow when AOT is operating.

Ultrasound has a clear impact on biofilm formation under the conditions tested within the scope of this study. It has the greatest impact on biofilm on titanium plates.

6. Future work

There is a difference in the amount of biofilm on SS compared to PS. It could be interesting to investigate if there is a difference in the type of adhered bacteria. This information is partly available on the agar plates. However, no conclusion was drawn on the exact species. This information could be valuable in order to do recommendation of material for different applications and locations, as well as cleaning methods.

As the performance of AOT is different in the first test compared to the second, the test should be performed again. A recommendation would be to perform the Crystal Violet analysis, in parallel with another analysis. Preferably this should be a quantitative and qualitative analysis, where the exact amount of biofilm is defined for the plates; this way the reliability of the Crystal Violet method could also be defined.

It would also be interesting to do the test on a medium with higher concentration of bacteria, for example metal working fluid or water with a higher concentration of bacteria. This way the possible impact of AOT would be more evident. Another way to clarify this would be to run the test for longer time spans, *i.e.* longer than 20 days.

The analytical method is time consuming; it takes up to 30 minutes to analyze one plate. An improvement of the method would be to immerse the plate in ethanol and then let some of the ethanol evaporate, and thereafter do the spectrophotometric analysis. Besides the time saving the effect of dipping would also be minimized and consequently the measurement error. On the other hand, this would mean a large usage of ethanol and the cost of the analysis would increase. To solve this, the size of the plates could be smaller. If the size of the plates were to be a fourth of the size of these plates, the test would still be on macro scale and the usage of ethanol would decrease. However, smaller plates would mean larger impact of measurement errors.

As described in section 4.8, AOT might influence the rate of biofilm formation, thus it could be useful to do more tests to define whether this is true or not.

The focus of this thesis has been on biofilm formation on heat exchangers. During this study there has not been any test on any heat exchanger; it would be valuable to do such a test. The method to analyze the results does not have to be Crystal Violet rather it should be pressure drop, or a combination of both methods.

Even if the amount of existing coatings on the market is limited, there are some available. To do a comparison between the performance of AOT, ultrasound and a common coating would be fruitful. The test could be realized in cooperation with companies producing or testing these types of coatings.

The bucket used in the ultrasound test was considerably small, which makes the system similar to an ordinary ultrasound cleaner system. Therefore, the results are expected. If the tests were performed on larger systems and the ultrasound would be placed directly on a heat exchanger the results would be more interesting and relevant. As ultrasound is used to all types of impurities, and crystalline fouling is the most common problem in cooling water systems, it would be valuable to see whether ultrasound cleans these types of impurities as well. If this test is to be realized there must be another analyzing method than Crystal Violet.

Tests to optimize the frequency and intensity in order to remove all bacterial growth and for optimal energy consumption should also be done.

No test to see if ultrasound had any effect on already existing biofilm was realized, in future work these experiments should also be performed.

The experimental set up for the AOT-tests compared to the ultrasound tests are considerably different and thus also the conditions for biofilm formations to occur. Therefore, it is difficult to compare the two methods. From this study, it appears like ultrasound is more effective against biofilm formation. However, to be able to compare the methods they should be performed under the same conditions.

During this study, it was not possible to do an economical research due to time constraints. For future work it would be fruitful to do such an evaluation; whether having an AOT or an ultrasound in a heat exchanger system would be feasible or not.

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Appendix A: Composition of 254 SMO Stainless steel

Element	**Composition[%]**
Carbon	Max 0.02
Manganese	Max 1
Phosphorus	Max 0.03
Sulfur	Max 0.01
Silicon	Max 0.8
Chromium	19.5-20.5
Nickel	17.5-18.5
Molybdenum	6-6.5
Nitrogen	0.18-0.20
Cu	≈0.02-0.7
Iron	Balance

Appendix B: Pictures of the actual biofilm on the plates.

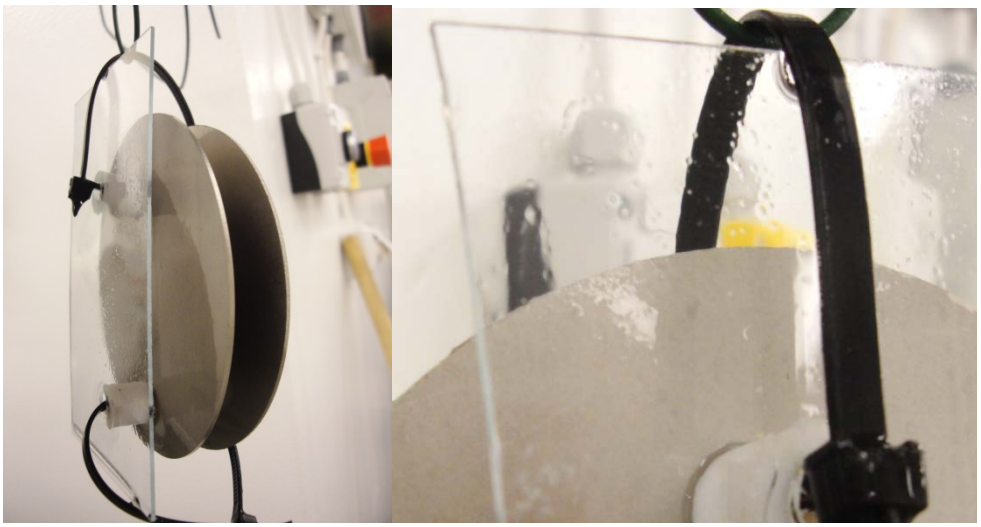

Figure B-1: Pictures taken from plates that had been immersed 10 days when AOT was operating. The second is another picture taken from the plates zoomed in. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

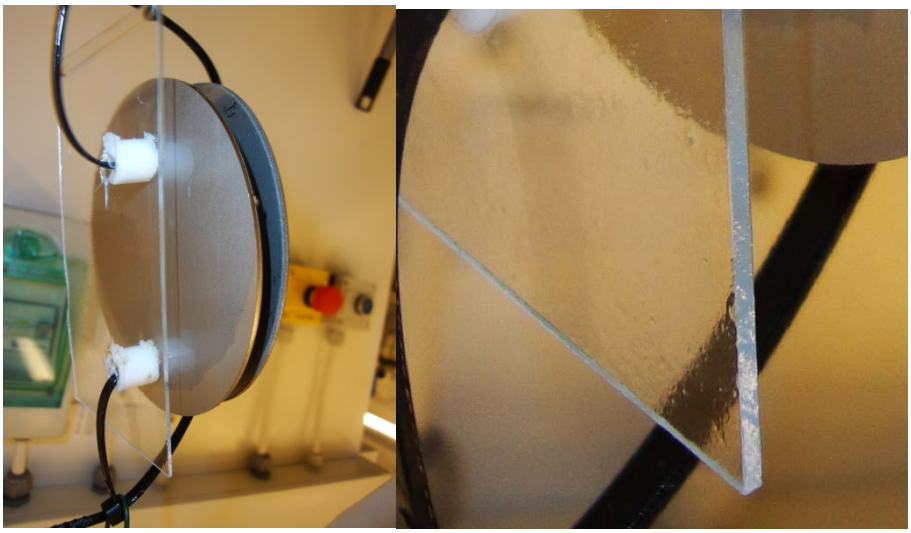

Figure B-2: Pictures taken from plates that had been immersed 10 days when AOT was operating. The second picture is a part of the first picture zoomed in. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

Figure B-1: Pictures taken from plates that had been immersed 10 days in the bucket when ultrasound not was operating. The second picture is a part of the first picture zoomed in. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

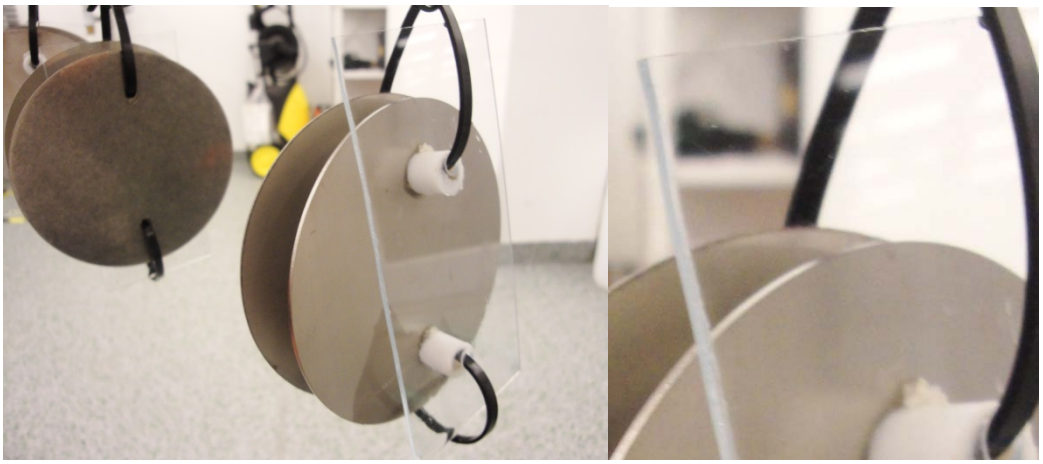

Figure B-2: Pictures taken from plates that had been immersed 10 days in the bucket when ultrasound was operating. The second picture is a part of the first picture zoomed in. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

Appendix C: Pictures taken of the agar plates

The photographs below, on each series, are from the agar plates that have been incubated in 22^0 C, the pictures below are the ones which have been incubated at 37^0C. The two left pictures are taken with an iPhone 5 camera, on the plates from the reference test for ultrasound, where “lilla” indicate the reference tank, and “Stora” refers to the bigger tank- where the second AOT- test was performed. The photos on the right side are taken with a Sony Cyber-shot DSC- W290 camera on the plates from the second test with ultrasound operating.

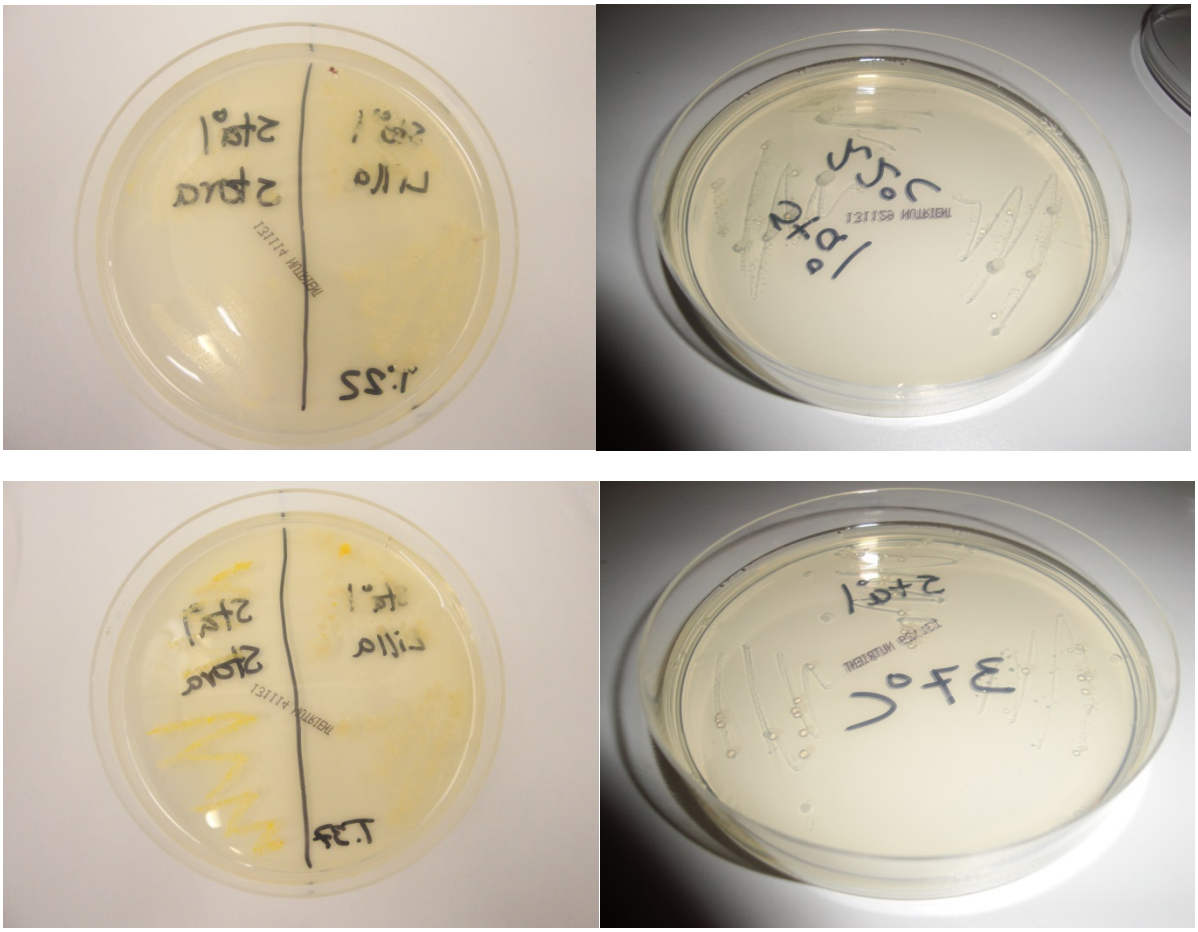

Figure C-1: Bacteria taken from the stainless steel plates that has been swiped over agar plates and incubated during 2 days.

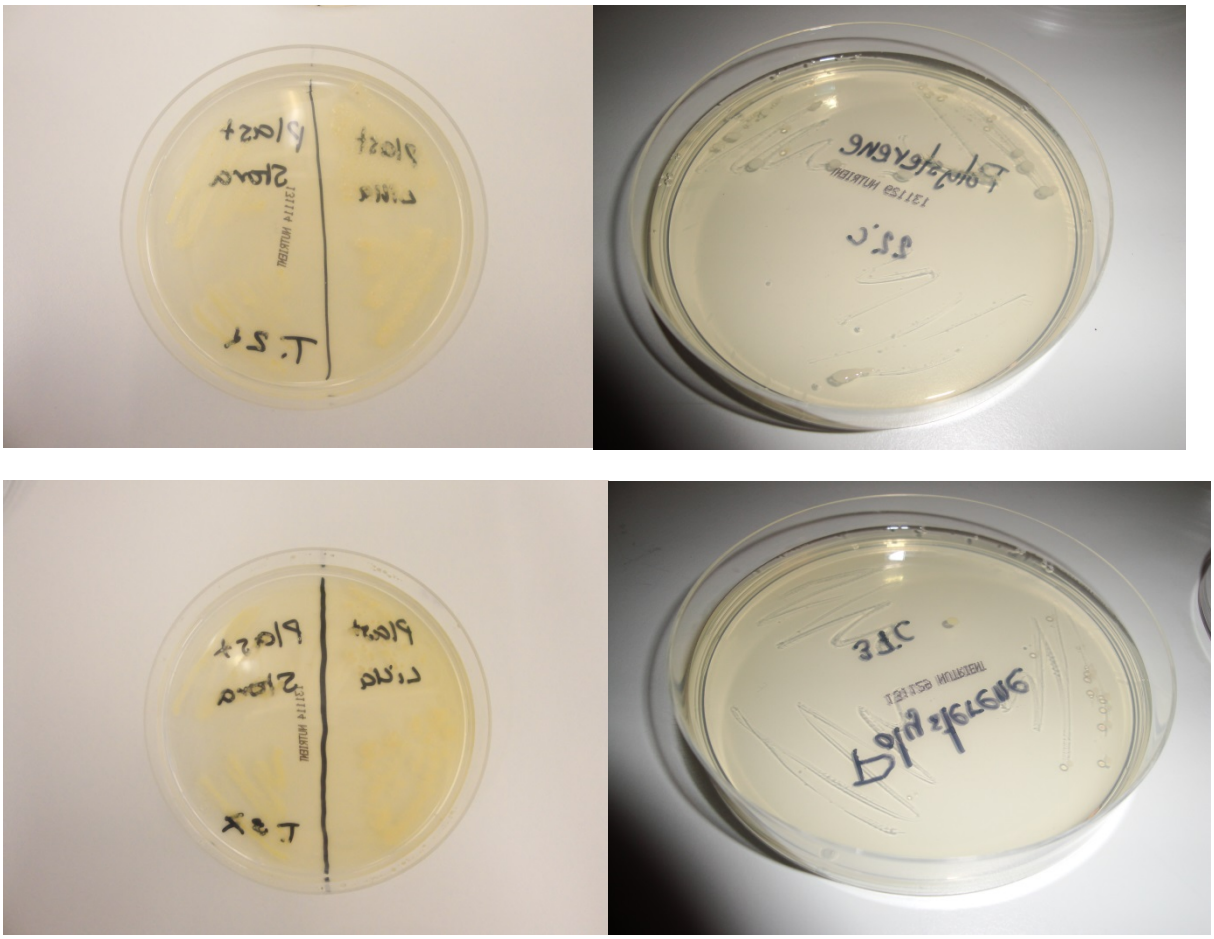

Figure C-2: Bacteria taken from the polystyrene plates that have been swiped over agar plates and incubated during 2 days.

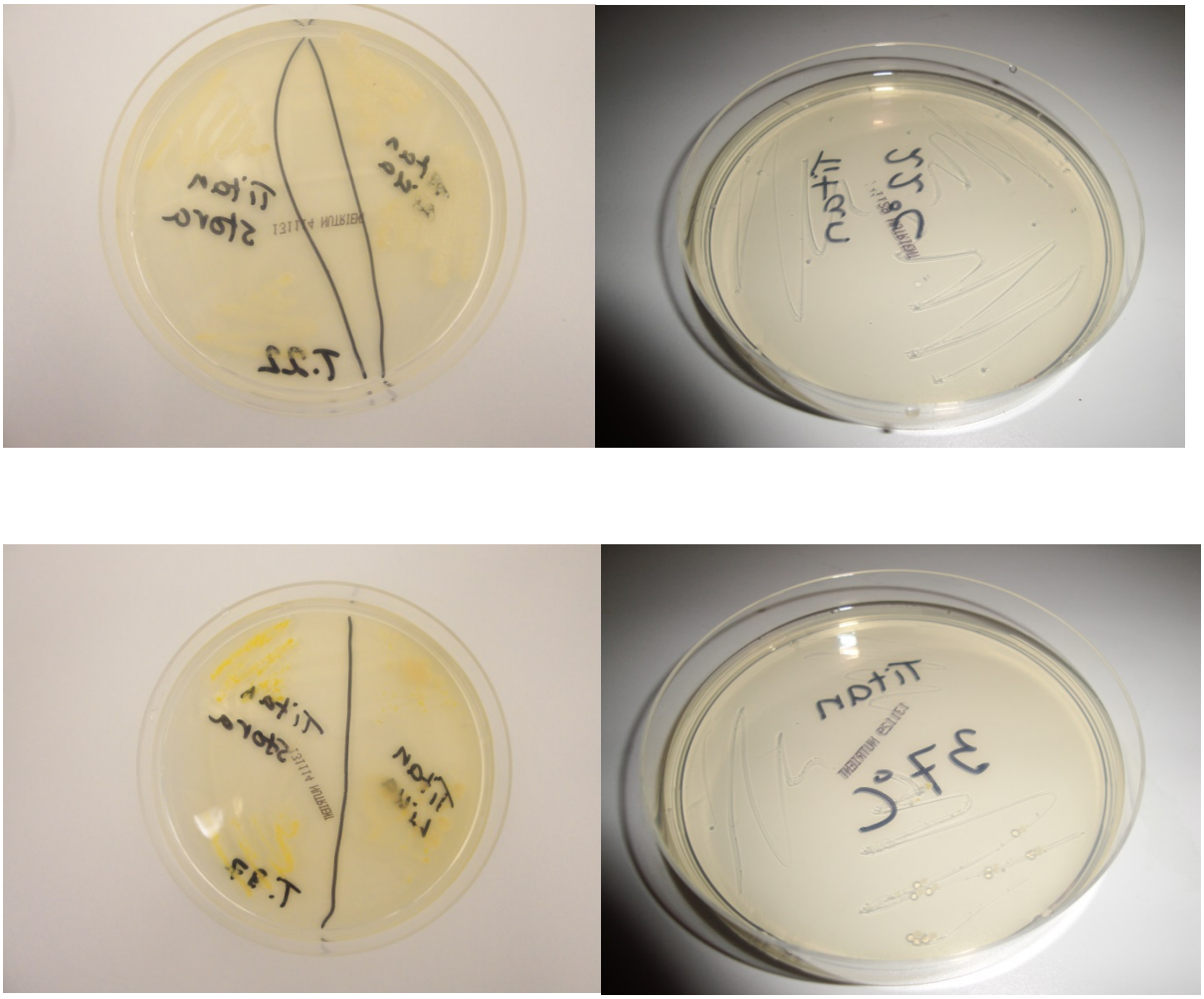

Figure C-3: Bacteria taken from the titanium plates that have been swiped over agar plates and incubated during 2 days.

There is a clear difference in the bacterial growth from the photos from the left side compared to the right side, i.e. there is a less bacterial growth on the plates when ultrasound was operating. Nevertheless, there are bacteria on the agar plates and thus there must have been bacteria on the plates in the bucket. There is also less bacterial growth in the bigger tank at 22^0C, as can be seen from photos on the left side above.

Figure C-4 and C-5 show the agar plates with bacteria from the plates in the ultrasound test.

Figure C-4: The agar plates that swabbed the plates during ultrasound test. These plates had been incubated in room temperature for 9 days. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

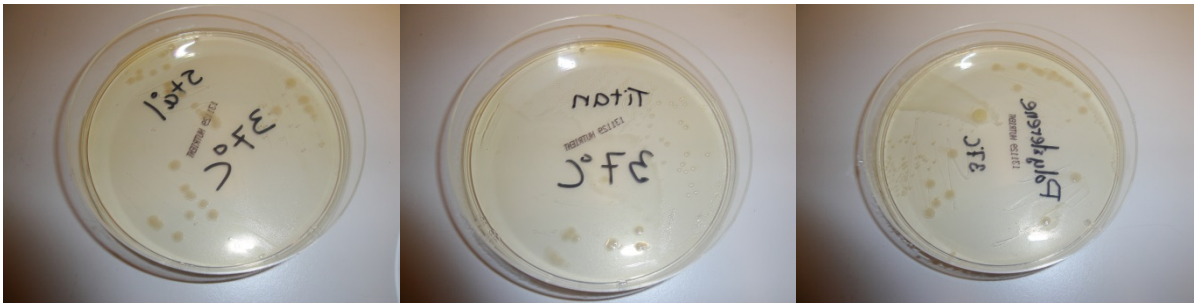

Figure C-5: The agar plates that swabbed the plates during ultrasound test. These plates had been incubated in 37^{0}C temperature for 9 days. The pictures are taken with a Sony Cyber-shot DSC- W290 camera.

As can be seen from the picture, most bacteria were produced in room temperature. Another interesting observation is that there is little bacterial growth on the titanium agar plate at 22^{0} and that the colonies are yellowish, whereas on steel and polystyrene there are also white-transparent colonies.

Appendix D: Statistical results from the different experiments.

First test with AOT

Table D-1: Calculated t-value in comparison with t-Crititcal. The table also shows the different amount of produced biofilm for different combinations.

Plate types	Material	Calculated t-value	t-Critical	Percentage increase/decrease in biofilm production (Second value/first value)
Plates from the first 10 days without AOT	Stainless steel-Titanium	2.43	2.78	83 %
	Polystyrene-Titanium	4.82[1]	2.78	180 %
	Polystyrene-Stainless steel	1.34	2.78	53 %
Plates from the last 10 days with AOT	Stainless steel - Titanium	2.59	2.78	-87 %
	Polystyrene-Titanium	7.37[3]	2.78	-91 %
	Polystyrene- Stainless steel	0.876	2.78	-29 %
Plates that has been 20 days in the tank	Stainless steel - Titanium	2.01	2.78	33 %
	Polystyrene-Titanium	8.77[3]	2.78	160%
	Polystyrene- Stainless steel	3.39[2]	2.78	93%
With and without AOT (Comparison for the two sets of plates that were 10 days in the tank)	Titanium	7.79[3]	2.78	-98%
	Stainless steel	2.74[≈5]	2.78	-75%
	Polystyrene	4.27[5]	2.78	-46%

[1] For the titanium plates vs. polystyrene plates placed for 10 days without AOT, 10 days with AOT and the three plates that were placed in the tank during the whole test period have a higher $|t\ Stat|$ than $|t\ critical|$.
[2] For the stainless steel plates vs. polystyrene plates placed in the tank during the whole test period has a significantly difference in biofilm production as $|t\ Stat|$ than $|t\ critical|$.
[3] There is a significant difference in biofilm production when AOT is operating or not as $|t\ Stat|$ than $|t\ critical|$.

Second test with AOT

Table D-2: Calculated t-value in comparison with t-Crititcal. The table also shows the different amount of produced biofilm for different combinations.

Time limit	Material	Calculated t-value	t-Critical	Percentage increase/decrease in biofilm production (Second value/first value)
Plates from the first 10 days without AOT	Titanium-Stainless steel	8.29[6]	2.78	-98 %
	Titanium-polystyrene	8.60[4]	2.78	-94 %
	Polystyrene- Stainless steel	4.88[6]	2.78	170 %
Plates from the last 10 days with AOT	Stainless steel-Titanium	5.29[6]	2.78	-97 %
	Polystyrene-Titanium	1.75	2.78	-94%
	Polystyrene- Stainless steel	0.52	2.78	120 %
Plates that has been 20 days in the tank	Stainless steel-Titanium	3.98[6]	2.78	-53 %
	Polystyrene-Titanium	3.07[6]	2.78	-40 %
	Polystyrene- Stainless steel	1.15	2.78	28 %
With and without AOT (Comparison for the two sets of plates that were 10 days in the tank)	Titanium	-	-	-
	Stainless steel	1.43	2.78	-20 %
	Polystyrene all numbers	0.95	4.3	74 %
	Polystyrene without the extreme large number	0.122	3.18	- 2 %

[4] All the combinations of plates showed significant difference from each other as |t Stat| than |t critical| during the first 10 days of testing. For the other test period titanium vs. stainless steel have significantly difference in production as titanium vs. polystyrene for 20 days.

Test with ultrasound

Table D-3: Calculated t-value in comparison with t-Crititcal. The table also shows the different amount of produced biofilm for different combinations.

Plate types	Material	Calculated t-value	t-Critical	Percentage increase/decrease in biofilm production (Second value/first value)
Plates from the reference test	Stainless steel- Titanium	0.90	2.78	-9 %
	Polystyrene- Titanium	2.9[5]	2.78	-13 %
	Polystyrene- Stainless steel	0.39	2.78	-4 %
Plates from the test when ultrasound was operating	Stainless steel- Titanium	12.1[7]	2.78	-
	Polystyrene -Titanium	4.84[7]	2.78	-
	Polystyrene-Stainless steel	1.74	2.78	40 %
Difference in biofilm production between reference and ultrasound test	Titanium	27.3[7]	2.78	-100 %
	Stainless steel	6.67[7]	2.78	-68 %
	Polystyrene	14.5[7]	2.78	-78 %

[5] There is a significant difference in production of biofilm on polystyrene vs. titanium plates, as │t Stat│ is larger than │t critical│. There is also a difference on the biofilm formation on the titanium vs. stainless steel plates that were immersed in the tank during 20 days. Additionally, the difference of biofilm production when ultrasound was operating compared to the reference is significant for all material types.

Appendix E: Number of bacteria in the water.

Table E-1: Number of bacteria in the water, measured with dip-slide method. Results taken from the first AOT-test.

Hours of incubation	Concentration of bacteria [TTC/ml]
72 (Thursday 24 October to Monday 28 October)	10^4-10^5
72 (Monday 28 October to Friday 1 November)	10^3-10^4
72 (Monday 4 November- Friday 8 November)	10^4-10^5
72 (Friday 8 November- Monday 11 November)	$\approx 10^4$

Table E-2: Number of bacteria in the water, measured with dip-slide method. Results taken from the second AOT-test.

Hours of incubation	Concentration of bacteria [TTC/ml]
72 (Friday 15 November to Monday 18 November)	0[6]
72 (Tuesday 19 November to Friday 22 November)	10^4
72 (Monday 25 November to Thursday 28 November)	$<10^3$
96 (Thursday 5 December to Monday 9 November)	0[1]

Table E-3: Number of bacteria in the water, measured with dip-slide method. Results taken from the second AOT-test.

Hours of incubation	Concentration of bacteria [TTC/ml]
72 (Tuesday 19 November to Friday 22 November)	10^4
72 (Monday 25 November to Thursday 28 November)	$<10^3$
72 (Friday 6 December to Monday 9 November)	$<10^3$

[6] The zero results probably come from a measurement error. Or there was something wrong with the dip-slides, or the tests were not performed correctly.

Appendix F: Analysis of Crystal Violet staining method

The effect of dipping

Due to the different results from the first and the second AOT test, an analysis of the effect of dipping was performed. For ten days ten polystyrene plates was submerged in the same tank as during the AOT experiments.

Afterwards, each plate was stained in Crystal Violet solution during 45 minutes. Thereafter, the plates were dipped in distillated water in pairs of two replicates. The first two replicates was not dipped at all, the second pair 4 times, the third pair 8 times, the third pair 12 times and the last pair 16 times. The same procedure was performed for ten other plates that had not been submerged in the tank water; these plates were used as blanks. The plates were then dried and the color desorbed with ethanol, as for the plates in previous experiments. The Crystal Violet-ethanol solution was then analyzed in a spectrophotometer. The results from the test are shown in the figures below.

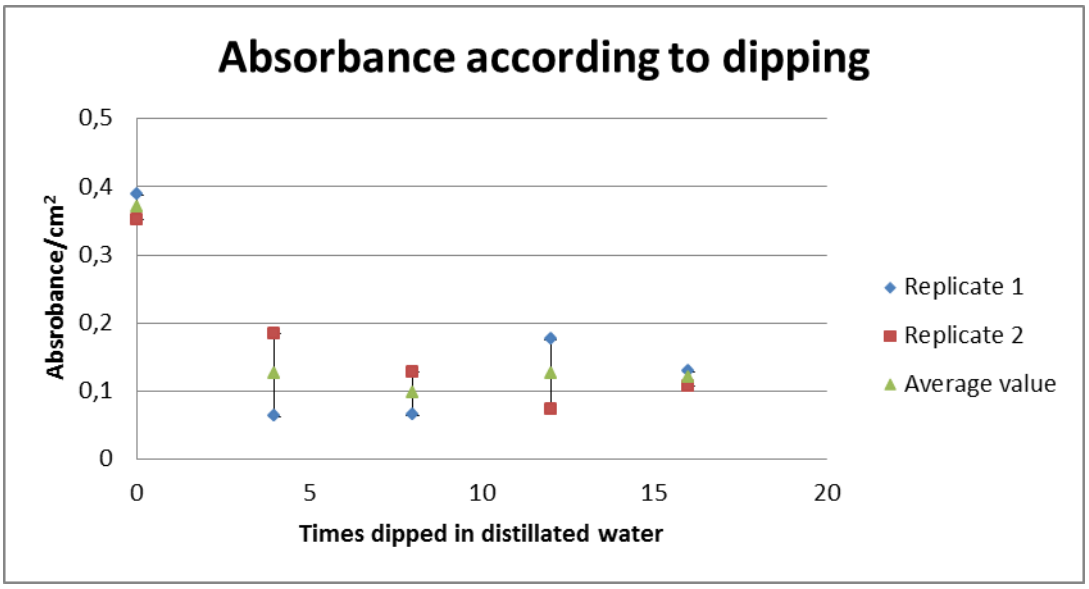

Figure F-1: The figure shows the absorbance of the plates submerged in the tank according to dipping. The result from the correlated average blank has been removed from all results.

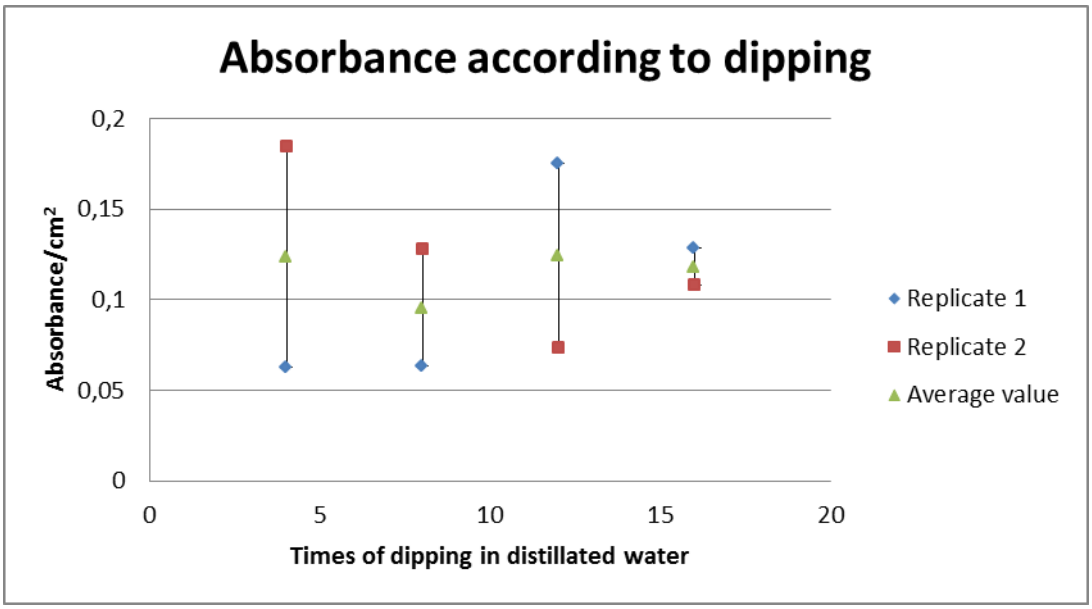

Figure F-2: The figure shows the absorbance of the plates submerged in the tank according to dipping. The two values from the plates that were not dipped are not shown.

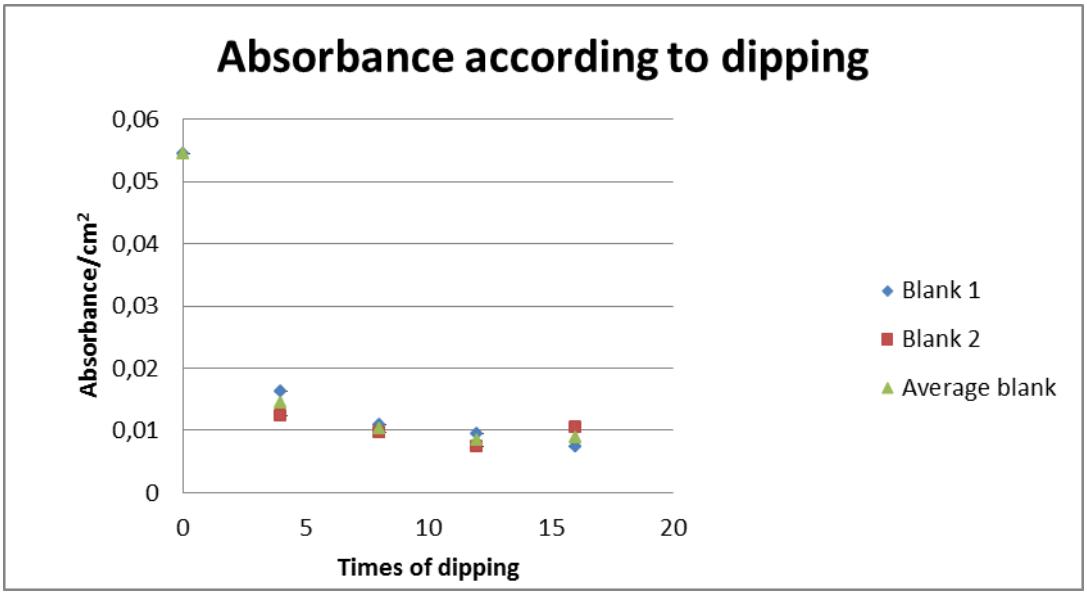

Figure F-3: The figure shows the absorbance of the blank results according to dipping.

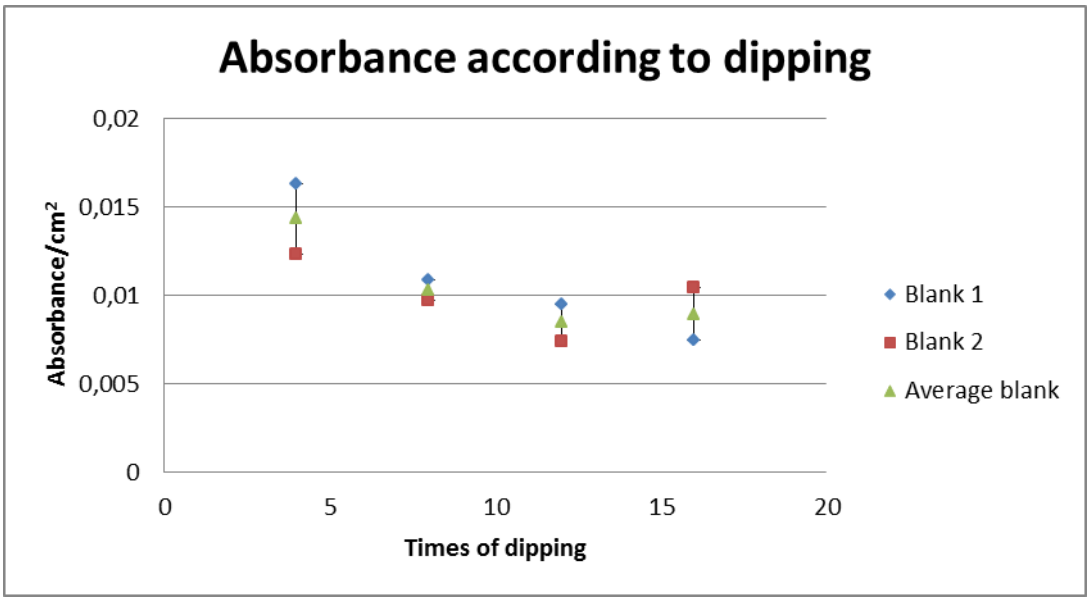

Figure F-4: The figure shows the absorbance of the blank result according to dipping. The two values from the plates that were not dipped are not shown.

Figures F-1, F-3 and F-4 show a decrease in absorbance according to dipping. The biggest decrease is shown from when the plates are not dipped until they are dipped four times. After that the correlation is rather straight linear, see figure F-2. Another aspect is that the results from replicate 1 and replicate 2 are fairly different. The results from replicate 2 are decreasing with the times of dipping (except from the very last value), while results from replicate 1 do not show this behavior.

Besides the absorbance, it can be seen from figure F-2 that the standard deviation is smaller for the plates that was not dipped in water compared to the ones which were. This emphasizes the theory that dipping results in a higher measurement error.

Sources of errors

The plates were bound to each other with cable ties when they were in the tank. After they had been dried, they were separated from each other and placed by themselves in the staining tank. Some of the plates fell into water in the small bucket when they were separated from each other. As the biofilm on the plates were thin this could have caused reduction in biofilm. The plates that fell were the two plates that were dipped four times and one that was dipped eight times.

The formation of the biofilm could differ on the plates, even if they should not, as all the previous tests shows fairly the same results independently on position.

There was a big difference in the amount of biofilm within every plate pairs, this could be seen on the plates after staining, see figure F-4. This caused the big difference in absorbance for these plates. The reason for this is most probably that the plates fell.

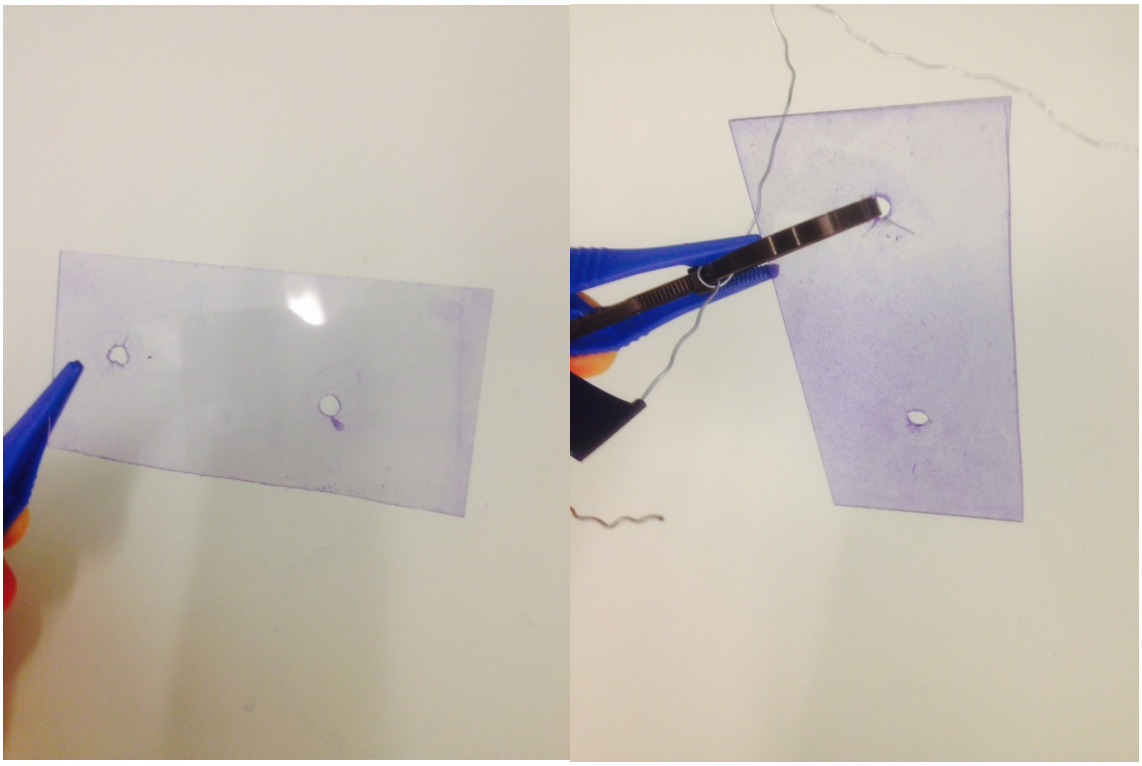

Figure F-5: The difference in biofilm for the two plates that had been dipped four times. As can be seen in the figure the plate on the right side has more biofilm on the lower part of the plate than the left plate.

Discussion

The difference between the replicates can attributed to that there actually was a difference in the biofilm on these plates, or that the act of dipping had an impact, i.e. the dipping was performed differently on the one replicate compared to the other. The different results for the pair that were dipped 4 times is most probably because the fell, causing in different amount of biofilm on these.

Conclusions

The general trend is that the forms of the lines in the figures are asymptotic; they reach a final value. This means that dipping after a certain amount of times does not have an impact of the result. However, the final values might be very small, as in this example, and therefore it can be difficult to see a difference between to different experiments and consequently draw conclusions. Another important aspect is the measurement error that increased with the times of dipping.

Appendix G: Other methods used in other applications to reduce fouling

All over the academic world and in industries it exists several types of antifouling coatings that can be used in different applications. Some of these are available on the market, others are under development. Four examples of these are listed in table G-1 below.

Table G-1: Antifouling coatings for other applications than heat exchangers.

Coating	***Mechanism***	***Performance***	***Applications***
Carbon Nano Tubes (CNT)	Large surface area High hydrophobicity. Microorganisms adhere softly to the surface and release from the surface under flow [81][140].	Good results in laboratory scale[81]. 150 % better removal of *Ulva linza*, compared to plain PDMS(polydimethylsiloxane)[140].	Hulls Fish nets Power inlets.
Polyflouroethylene (PFTE)	Low surface energy; 24.7 mN/m [73].	EN(electroless nickel)-PFTE showed a 97% less fouling than plain SS [73].	Several
Polyethylene glycol (PEG)	Low interfacial energy Water interfacial energy< 5 mJ/m^2 Resulting in less protein and cell adhesion [65].		
Changes in nano-topography	The nano- scaled topography makes it difficult for bacteria to obtain good contact with the material [141].	50 % reduction in cell attachment [141].	Medical devices