Cleaning technology in high temperature food processing

-A Literature Review

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Abbreviations:

AAS - Atomic absorption spectroscopy
AFM - Atomic Force Microscopy
CIP – cleaning in place
COD - chemical oxygen demand
ESCA - Electron Spectroscopy for Chemical Analysis
FE-SEM - Field-Emission Scanning Electron Microscope
FRAP - Fluorescence recovery after photo bleaching
HPLC - high performance liquid chromatography
MFGM - milk fat globule membrane
pI – isoelectric point
QCM-D - Quartz crystal microbalance with dissipation
SEM - Scanning Electron Microscope
SMUF - Simulated milk ultra-filtrate
TEM - Transmission electron microscopy
UHT - Ultra high temperature
WPC - Whey Protein Concentrate
WPI - Whey Protein Isolate
XPS - X-ray Photoelectron Spectroscopy

Subscripts

A area
A_XXX and Å_XXX absorbance at a specific wavelength
C constant
c velocity of sound
c_i concentration of element i
D diameter
D_i diffusion coefficient of element i
E binding energy
e energy flux vector
E_r rate of reaction
f friction factor
F thickness of reaction front
h convective heat transfer coefficient
h distance between two surfaces
H Hamaker constant
hν photon energy (where h is the Planck’s constant and ν the photon frequency)
J mass transfer flux
k Boltzmann constant
k_A first order rate constant
k_m is the mass transfer coefficient
k_t thermal conductivity coefficient
L distance
M mass
n overtone number
p pressure
r and R radius
R ideal gas constant
r thickness of composite
R_F fouling resistance
r_i rate of reaction
T temperature
U overall heat transfer coefficient
V volume
w mass rate of flow
X unreacted fouling
Y swelled intermediate fouling
Z final removable units (section 7.2.1)
Z impedance (section 8.3)
z valence

1/κ Debye screening length
β is the mass transfer coefficient
γ surface tension
η viscosity
θ contact angle
ξ kinetic constant
ρ density
φ volume fraction
ψ surface potential
1 Introduction

Milk is a diverse raw material. Milk for human consumption mainly comes from cow, goat, sheep and buffalo, where the cow milk represents 84% of the world’s milk consumption. The milk consumption in the world is large, and in 2009 the consumption of milk and milk products, also including production of feed, was 703 million tons [1]. In Sweden the produced amount of milk was 2.85 million tons in 2011 and the largest part of the production is the milk for direct consumption together with the ferment milk products like yoghurt (Figure 1) [2].

![Figure 1 The production of milk products from the Swedish cow milk. Adapted from the Swedish Dairy Association [2]](image)

Milk is produced in the mammary glands in the cow udder and is released upon contraction. The milk is sterile as long as it is inside the udder in a healthy cow. It is during milking and transport to the dairy that the milk is exposed to microorganisms that can contaminate the milk. Not all microorganisms can digest lactose, which is the main digestible carbohydrate source in milk. Milk is also deficient in iron, which for many bacteria is necessary for growth, but there are those that do survive and thrive. Some of these organisms can cause disease and in severe cases be fatal.

Milk and milk products have to be safe to be allowed into the market for human consumption and since raw milk may contain several bacteria, it has to be processed to assure safety. Milk is therefore heated to a certain temperature in order to kill off the microbial growth and to deactivate some of the enzymes that otherwise can degrade the lipids and proteins, decreasing the shelf life of the product. The processing of raw milk can differ between different dairies, but the main aim to produce a safe high quality product is the same.

Milk is usually standardized by mixing skim milk and cream to obtain the desired fat content of the product. The standardized milk can then either be pasteurized, i.e. heat treated to about 75°C, or treated at a higher temperature to produce a product with a longer shelf life. The raw milk is filtered when it arrives to the dairy and is as a second step either cooled or rapidly heated before cooling. A rapid heating can neutralize some microorganisms, which allows the pasteurization temperature to be slightly lower. The milk is then separated into one high fat or
Introduction

cream fraction, which partially is intended for high fat products, and skim milk. The cream that is intended for low fat product is homogenized before remixing with the skim milk to the desired fat content. The homogenization is needed to prevent phase separation with a cream layer on top of the skim milk [3].

For countries where the milk consumption is high as it used as a beverage, the off flavor taste that can be caused by ultra-high temperature (UHT) treatment is less desirable. In those markets, the pasteurized milk is popular and the processing time after standardization is short. The milk is pasteurized for 20 seconds at approximately 75 °C and is then packaged and stored dark and cold. The milk processed with UHT sterilization is first going through a pasteurization process and then the sterilization takes place at approximately 140°C. The sterilization can be performed with both direct and indirect heating. Indirect heating with the aid of a heat exchanger, require the milk to be homogenized. The gas dissolved in the liquid phase needs to be removed before the heat treatment in order to decrease the deposit formed on the walls of the heated tubes (or plates). UHT milk is packaged aseptically to prevent recontamination after treatment and is then stored for a certain amount of days at the dairy in room temperature to assure that no microbial growth occur in the product [3, 4].

1.1 Challenges for heat processing of milk

While reaching the fatal temperature for bacteria, the denaturation temperature of several proteins is also reached and these proteins tend to aggregate and precipitate and or deposit onto the heated surfaces, fouling (Figure 2).

Milk has the health benefit of providing the consumer with important minerals such as calcium. However, the high content of calcium also creates one of the issues with fouling of heated surfaces. The solubility of calcium phosphate has an inverse heat dependency and the solubility of the mineral will therefore decrease with elevated temperature. Milk processing therefore gives a fouling containing both proteins and minerals. For temperature below 100 °C the fouling on heated surface mainly contains protein (~60 %), whereas a change occur for temperatures above 120 °C, above which the predominant constituents are inorganic compounds (~80 %).

Figure 2 Fouling from heat-treated skim milk in a stainless steel canal.
The deposit of protein and minerals on the heated surfaces is not so severe that it will decrease the benefits of consuming milk and milk products by depleting them from nutrient. One of the main draw-backs with the deposit is rather increase in the heat transfer resistance with the buildup of fouling. The deposit is also a very nutritious habitat for thermophile bacteria and spores from the unprocessed milk. One solution to assure that enough heat is transferred through the deposit to the milk is to increase the temperature of the heated surface, but this could both increase the fouling layer and is inefficient from an energy consumption perspective. Cleaning of the equipment is done when the difference in temperature between the heating media and the outlet milk exceed a certain limit.

Up to 6 hours per day is needed for sufficient cleaning of certain parts of the dairy process equipment such as sterilizers. The water consumption during cleaning is high, approximately 0.5 to 5 liter per liter milk processed [5]. Cleaning is often performed in a closed loop (Cleaning in place, CIP) with no other possibility to detect the result but to open the equipment. The mechanisms of both fouling and cleaning have been studied for several decades, but fundamental understanding of the mechanisms behind cleaning of milk fouling is still lacking. This is especially true for the mineral rich fouling formed during elevated temperatures. The reason is that it is difficult to study this process due to the lack of in-line measurements and the difficulty to produce this particular fouling on a small laboratory scale.

With a better understanding of the mechanisms behind cleaning of different fouling systems, a tailored cleaning protocol could be designed and used. Such cleaning programs could help save energy, water and chemical consumption in the large dairy industries, thus provide input towards an environmentally friendly, and safe food processes.
Milk composition and properties

2 Milk composition and properties

Milk from cows consists of approximately 87 % water. The remaining 13 % dry matter components are divided among: carbohydrates, proteins, fat and inorganic material [6]. In addition to milk; butter, cheese and milk powders are common dairy products consumed in the world [1]. The concentration of the different components in milk can vary between mammals of different species (Table 1), but also between different breeds. The health and the environment for a cow are of great importance for the properties of milk, as well as the stage of lactation and the breed. Seasonal changes occur in for example fat and protein concentration, which prove a challenge and force adaptation in the dairy industry [7].

Table 1 Difference in milk composition between species g 100 g⁻¹ [8]

<table>
<thead>
<tr>
<th>Species</th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>3.9</td>
<td>3.2</td>
<td>4.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Goat</td>
<td>4.5</td>
<td>3.2</td>
<td>4.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Sheep</td>
<td>7.2</td>
<td>4.6</td>
<td>4.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Buffalo</td>
<td>7.4</td>
<td>3.8</td>
<td>4.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

For this chapter the constituents of milk will be considered in more depth. The properties of the constituents, when the temperature and pH change, are the reason that dairy products are susceptible to fouling during the industrial process.

2.1 Carbohydrate and lipid chemistry of dairy products

Lactose

Together with fat and proteins, carbohydrates are main components in milk, where lactose (4-O-β-D-galactopyranosyl-D-glucopyranose) is the most abundant sugar in milk. It is a disaccharide composed of Galactose and Glucose (Figure 5). Lactose is important for the newborn, due to the high energy content. The concentration in milk is approximately 4.6% w/w [9]. The lactose concentration is higher early in lactation and then reduced with time due to the decreased need for the energy supplement to the newborn. The synthesis of lactose in the mammary gland can be regulated by the whey protein α-lactalbumin since its presence changes the specificity of the enzyme Galactosyltransferase [7].

Figure 3 Lactose is a disaccharide formed by galactose and glucose.

During heat treatment Lactose will cause browning and off-flavor due to the Maillard reaction (section 3.3).
Lipids

The predominant lipids in milk are the triglycerides (98%, Figure 6), but milk also contain phospholipids, cholesterol and low concentration of fat soluble vitamins [7].

The fatty acid composition can vary between batches of milk and differences in the amount of saturated and unsaturated fatty acids can be seen at different stages of lactation as well as varying with season [7]. Milk fats are generally more saturated during the winter and less saturated during spring and summer. The fatty acid composition also differs depending on the feed, which is another cause for the observed changing milk and dairy product properties with season.

![Figure 4](image)

Figure 4 Schematic image of a triglyceride with the glycerol backbone and three fatty acids. R is a hydrocarbon chain and the saturation of these will determine some of the properties of the fat in the milk.

The vitamin group β-carotenoids for example are soluble in the fat fraction of milk and give the yellow color to butter. The concentration of β-carotenoids is feed dependent, hence also the color of certain dairy products.

The milk fat globule

Most of the lipids found in milk are located in spherical globules (0.1 – 20 am) and milk can therefore be regarded as an emulsion of oil in water [7]. The phospholipids, corresponding to no more than 1% of the total amount of the milk lipids, are very important to the microstructure of these fat globules. The phospholipids create the membrane structure surrounding a triglyceride core [10, 11]. The main structure holding the globule together is a monolayer of polar phospholipids surrounded by a phospholipid bilayer (Figure 7). The outer membrane of the globule consists of not only phospholipids, but also of lipoproteins, proteoglycans, trans-membrane proteins, and also of water bound to the surface. The protein in the fat globule membrane account for approximately 1.2 % of the total protein content in milk [6].

The polar outer layer of the fat globule prevents the oil phase to separate from the water phase, i.e. prevent creaming. During homogenization of milk, the size of the globules are decreased, thus the surface area is increasing. Since the phospholipid concentration in milk is limited, proteins from whey and the casein fraction cover a significant fraction of the milk fat globule. The interaction of protein and lipids on the fat globule, in addition to the smaller droplet size, make the homogenized milk less prone to phase separate (less creaming).
Figure 5 The milk fat globule membrane (MFGM) has a triglyceride core surrounded by first a monolayer followed by a bilayer of polar phospholipids. Proteins are additionally inserted into the bilayer of lipids, influencing the properties of the globule. Picture adapted from the work of Dewettinck and coworkers [11].

2.2 Inorganic salts in milk

The inorganic content of milk is in the literature usually referred to as ash and is a combination of different inorganic components (Table 2). Although some of the salts in milk, e.g. calcium phosphate, are associated with the casein micelles, significant amounts are still present in the aqueous phase (1/3 of calcium, ½ of the inorganic phosphate, 2/3 of the magnesium and 90% of the citrate) [12]. During the heat treatment of milk and the buildup of fouling, one of the main roles is played by the calcium phosphate.

Table 2 Mineral content of milk given in mmol/kg. The numbers are adapted from [12].

<table>
<thead>
<tr>
<th>Cations</th>
<th>Amount (mmol/kg)</th>
<th>Anions</th>
<th>Amount (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>17-28</td>
<td>Cl</td>
<td>22-34</td>
</tr>
<tr>
<td>K</td>
<td>31-43</td>
<td>PO4</td>
<td>19-23</td>
</tr>
<tr>
<td>Ca</td>
<td>26-32</td>
<td>Citrate</td>
<td>7-11</td>
</tr>
<tr>
<td>Mg</td>
<td>4-6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Calcium phosphate**

Calcium content in milk is high and is regarded as one of the main health benefits of milk consumption. The concentration of calcium phosphate in milk reaches the limit of super saturation and would not be soluble in the milk if it did not form complexes with other constituents in the milk.

Casein micelles are one of the main structures in milk and help to keep the calcium phosphate dispersed under supersaturated conditions. Not all calcium ions bind to an inorganic phosphor. A large part of the calcium in the casein micelle is bound to organic phosphor on the phosphoserine groups. The calcium phosphate interactions in the casein micelle is as will be discussed further below, important for the stability and properties of the micelle [12].

The micelles release lipids and calcium phosphate slowly during digestion in newborn and children as well as in adults. The solution with casein will also help the secreting glands in the animal to maintain their elasticity and not getting calcified. The role of caseins is to promote calcification of bone tissue, but prevent calcification of soft in calcium saturated environments [13].

**Reverse solubility**

Upon heating, the calcium phosphate will precipitate and is therefore one of the main sources of fouling during the heat treatment of milk (Figure 8). The precipitate can under some conditions be re-dissolved if the solution with precipitate is sufficiently cooled, but when the temperature is high, above 90 °C, the precipitation is usually irreversible and cannot be reversed by cooling [7, 12]

![Figure 6](image-url) The dissolved concentration of calcium and phosphate decrease with an increase in temperature. Image adopted from [7] with data from [14].

The formation of insoluble calcium phosphate particles during heating in dairy process often lead to a decrease in pH [15].
Milk composition and properties

\[ \text{Ca}^{2+} + \text{H}_2\text{PO}_4^{-} \rightarrow \text{CaHPO}_4 + \text{H}^+ \]

A decrease in in pH will change the properties of the milk and the behavior of many of the proteins. During heat treatment above 100 °C, the pH of the milk will decrease linearly due to the changes that occur in the mineral balance [16]. Up to approximately 80°C this change in pH is reversible and will go back to the normal pH of 6.6-6.7 when the milk is subsequently cooled. However for higher temperatures, further changes will occur, involving breakdown of lactose to formic acid and this will irreversibly lower the pH of the milk. During heat treatment at higher temperatures, for example at UHT-treatment, the ionic calcium content in milk decreases by 10-20% [7].

2.3 Milk proteins

Proteins are one of the fundamental building blocks of life. Proteins consist of amino acids and the structure of globular proteins can be divided into substructures, where the amino acid sequence and the secondary structure will determine the folding into the tertiary structure. The configuration of one domain will influence the folding of the protein, and without correct folding, the protein can lose its original function. Changes in pH and temperature are examples on environmental changes that can drastically alter the properties of proteins. Not all of the proteins in milk have secondary structure, such as the caseins, and is therefore also less sensitive to heat and pH changes. Cow milk has an approximate concentration of 3.4 % proteins (Table 3), and as also observed for the other components in milk, seasonal changes occur due to feed and lactation stages.

<table>
<thead>
<tr>
<th>Caseins</th>
<th>Portion of milk proteins (%)</th>
<th>Isoelectric point (pI)</th>
<th>Denaturation temperature[7]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha_s)-casein</td>
<td>30.6</td>
<td>4.92-5.35</td>
<td>-</td>
</tr>
<tr>
<td>(\alpha_s)-casein</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\beta)-casein</td>
<td>30.8</td>
<td>5.77-6.07</td>
<td>-</td>
</tr>
<tr>
<td>(\kappa)-casein</td>
<td>10.1</td>
<td>5.20-5.85</td>
<td>-</td>
</tr>
<tr>
<td>Whey Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-lactoglobulin</td>
<td>9.8</td>
<td>5.1</td>
<td>74</td>
</tr>
<tr>
<td>(\alpha)-lactalbumin</td>
<td>3.7</td>
<td>4.2-4.5</td>
<td>63</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>1.2</td>
<td>5.13</td>
<td>87</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>2.1</td>
<td>-</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 3 The isoelectric point and the denaturation temperatures are important parameters for the denaturation and aggregation of proteins. Values for the common proteins in milk are given as a combination of data from [6, 8].

There are two main groups of proteins found in milk, whey proteins and caseins. The predominant protein group in dairy products is casein. Both caseins and whey proteins have colloidal dimensions when dispersed in the water phase of the milk [6]. Only a small fraction of the casein proteins will remain in monomeric form, but will form micelles with the average diameter of 140 nm [8].
2.3.1 Whey protein

Whey proteins are the second largest group of proteins in milk and correspond to 20% of the proteins in milk. The group of whey proteins includes; α-lactalbumin, β-lactoglobulin, serum albumin and immunoglobulin, where the β-lactoglobulin is the most abundant. The whey proteins are globular with a secondary structure that determines the folding of the proteins into a tertiary structure.

The β-lactoglobulin monomer has five cysteine residues of which four is forming disulphide bonds in the interior of the folded structure (Figure 10). Cysteine groups are very reactive and the fifth cysteine residue is available for interaction, if it is exposed when the protein is monomeric and the tertiary structure is partially unfolded [7].

![Figure 7 Tertiary structure of β-lactoglobulin showing the disulphide bridges, one of the contributing forces keeping the structure together in its native form [17].](image)

The structure of proteins is sensitive to pH changes and so also for β-lactoglobulin. Many proteins precipitate at the isoelectric point. β-lactoglobulin associate into different oligomer forms and exists as a dimer at pH 6.7 and as an octameric protein around the pI at 5.1 [15]. The aggregation of the protein has been reported to be irreversible above pH 8.6. High concentrations of calcium ions will also cause the protein to aggregate, which will lead to a synergistic effect at elevated temperatures since calcium has a reverse solubility to heat [8].

The α-lactalbumin has eight cysteine residues forming four disulphide bonds, and has no sulphuric group free for interaction. The tertiary structure of α-lactalbumin molecules is stabilized with one calcium ion, but the denatured α-lactalbumin do not form aggregates with casein without first reacting and forming aggregates with β-lactoglobulin [7, 8].

2.3.2 Caseins

The casein content of milk contributes with 80 % of the protein content in milk and 95 % of the caseins form casein micelles [6]. Casein is a group of four proteins; α_{s1}-casein, α_{s2}-casein, β-casein and κ-caseins. In contrast to most other proteins, the caseins do not have the ability to form stable secondary structures.
2.3.3 Casein micelle formation

Most casein in milk is assembled in casein micelles. The phosphorylated proteins in the micelles bind and form calcium phosphate complexes, within the structure. It is both the ability to deliver calcium at tissues such as skeletal parts and to prevent calcium phosphate precipitation in other tissues that makes casein an important constituent in milk. The formation and construction of the casein micelles has long been debated [13] and there have been two leading models; the coat-core model and the submicellar model.

Coat-core model

The coat core model suggest a micelle where αs1-casein and β-casein assembly in the core of the micelle and the glycosylated κ-caseins concentrated on the outside, forming a micelle with a more hydrophobic core and hydrophilic shell. The κ-casein has a negative charge, which apart from the steric repulsion invoked by the brush like κ-casein layer, prevents aggregation of the micelles by electrostatic repulsion. The outer layer on the micelle is challenging to directly visualize due to the low segment density and therefore the existence of a κ-casein shell has unambiguously been proven. Rennet for cheese making contains an enzyme, chymosin, that cleaves off the glucomacropeptide from κ-casein and thereby reducing the protruding “hairy” layer of the micelle, reducing the steric stabilization as well as the charge so that the casein micelles aggregate [18]. This process suggests that the stabilizing κ-casein layer do exist.

All casein micelle models agree on the importance of forming calcium phosphate complexes as means of holding the casein micelle construct together. For the coat core model, these are thought of as colloidal nano clusters cross-linking the a proteins to a gel [18]. The calcium phosphate interaction, together with the hydrophobic effect, i.e. due to the fact that portions of the proteins involved have limited aqueous solubility and therefore tend to accumulate in the core, is thought to be enough to gain the free energy needed to spontaneously form casein micelles [6].

So in this respect the micelle structure is a gel particle held together by calcium/calcium phosphate clusters and could be used to explain the changes in gel strength seen during heat treatment. The hydrophobic effect will increase during heating, up to a certain temperature. The reverse solubility of calcium phosphate could possible lead to precipitation and thereby decrease the calcium phosphate keeping the proteins together as the temperature rises. This has been observed experimentally and used as an explanation for the lowering in gel strength with increasing temperatures [18].

Submicellar model

The second model is the submicellar structure, where small sub micelles are aggregated into larger micelles. Although, the coat-core model is most accepted model to describe the casein micelle structure, the submicellar model has evolved and does include the coating of κ-casein on the outmost part of the structure.
Milk composition and properties

The submicellar model came before the coat-core model, in the beginning of the 1980\textsuperscript{th} and was introduced by the research groups of Schmidt and Walstra in Wageningen. Images taken by the scanning electron microscope gave a visual image with resemblance of a raspberry shaped micelle, which inspired the development of the submicellar model.

In a study in 2004 the casein micelles from skim milk was visualized (Figure 12) with Field-Emission Scanning Electron Microscope (FE-SEM) [19]. The authors suggest a more complex structure than the coat-core model, with protruding cylindrical objects. They pointed out the possibility of the micelles to have bicontinuous structure [19].

![Image of a casein micelle taken by a Field-Emission Scanning Electron Microscope. Scale bar is 200 nm [19.]](image)

**Figure 8** Image of a casein micelle taken by a Field-Emission Scanning Electron Microscope. Scale bar is 200 nm [19].

### 2.4 Conclusions on the milk composition

Milk is a diverse raw material, with several health benefits. Since milk is a natural product produced by animals, the animal feed and other seasonal changes influences the overall properties. The protein and mineral coexist in order to keep the high content of calcium dispersed and accessible to the consumer. Both protein and mineral contents in milk are sensitive to changes in temperature and pH. Heat denaturation of proteins and mineral precipitation is there for inevitable during the heat processing in the dairy industry.
3 Dairy fouling, produced during heat processing

Fouling is the collective word used to describe the unwanted deposits forming on a surface in process equipment. Fouling originates from the fluid in contact with the surface, in this case the dairy product. All milk attended for consumption needs to be processed with a heat treatment to fulfill the demands of long shelf life without product spoilage and to assure that there is no growth of pathogenic microorganism in the product.

Proteins are naturally sensitive to heat, and heat treatment can therefore cause proteins to unfold, aggregate or deposit due to the changes in the milk.

3.1 Temperature dependent changes in fouling composition and structure

Fouling formed during milk processing does not look the same throughout the process equipment. It differs drastically. For the cold, non-heated sections, some adsorption from proteins and minerals will occur. On the surfaces heated for pasteurization of the product, more extensive fouling will occur. Another drastic change in both component composition and in structure occurs again when reaching a temperature above 110 °C.

In 1968, Burton, made a distinction between different types of fouling generated from heating milk [20]. This classification system has since been used for fouling at pasteurization and sterilization temperatures. The two main types of fouling that are formed inside the dairy plant are named type A and B (Table 4).

Table 4 The temperature dependence in fouling composition and structure caused the division of fouling type A and type B in the late 1960th [20]

<table>
<thead>
<tr>
<th>Type</th>
<th>Protein</th>
<th>Minerals</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A (&gt;75 °C, max. 95-110 °C)</td>
<td>50-60 %</td>
<td>30-35 %</td>
<td>4-8 %</td>
</tr>
<tr>
<td>Type B (&gt;120 °C)</td>
<td>15-20 %</td>
<td>70-80 %</td>
<td>4-8 %</td>
</tr>
</tbody>
</table>

The type A fouling is the most common fouling from pasteurization at temperatures below 100°C. The fouling layer closest to the metal surface might be dense, but the characteristic feature of type A fouling is the soft, voluminous and white structure. At temperatures above 120 °C, the type B fouling is dominating and is characteristic by a grey, brittle and granular structure. The fat content is about the same as for the type A fouling (4-8%), but the mineral content rises from 30-35 % to approximately 70 %, whereas the protein content decreases from 50-60% to 15-20%. Whey proteins are dominating in the protein deposit in spite that they are only 15-20% of the proteins in milk.[20]
3.2 Colloidal forces controlling milk fouling

When trying to understand the build-up of fouling, knowledge of the different forces that control the interaction between the deposits and between the deposits and the surface are of large importance. The classical theory to predict colloidal stability is the DLVO theory, named after four scientists active in the 1940s (Boris Dejaguin, Lev Landau, Evert Verwey and Theo Overbeek). The joint theory of the four scientists model the interacting forces between particles in solution and hence the colloidal stability of the system. According to the DLVO theory, the stability is determined by the competition between attractive van der Waal forces and the repulsion from the electrostatic double layer forces between the charged particles [21].

The full thermodynamic approach involves estimation of the Gibbs free energy for the different components of the adsorption process. Although it is hard to quantify in absolute numbers, the contribution from different interactions can be estimated. According to Visser $\Delta G$ for the process can be expressed as

$$\Delta G = G^{LW} + G^{EI} + G^{AB} + G^{Br}$$

Visser chose to add the Lewis acid base interaction to explain the behavior of fouling [22]. The Brownian motion, $G^{Br}$, is often neglected for particles adhering to a surface since the contribution of $1 \text{kT}$ is very small in comparison to the other energies. Adsorption to the surface, e.g. stainless steel surface in the process plant, will occur spontaneously as long as the Gibbs free energy displays negative value.

3.2.1 Van der Waals attraction

Van der Waals forces include dipole-dipole, dipole-induced dipole and induced dipole-induced dipole interactions. The Gibbs free energy for the van der Waals attraction of particles is given by

$$\Delta G^{LW} = \frac{H \ast r}{L} = -2\gamma^{LW}$$

Where $r$ is the radius of the particle and $L$ is the shortest distance between the two surfaces in contact. $H$ is the Hamaker constant that depends both of the particle-particle pair interactions between the elements and the number atoms per unit volume [21].
Dairy fouling

Table 5 Hamaker constants for key components for fouling onto stainless steel. The constant given for metal is given without consideration of the oxide layer forming on top of the surface [22].

<table>
<thead>
<tr>
<th>Component</th>
<th>( H_{11} \times 10^{-20} ) J</th>
<th>( H_{132} \times 10^{-20} ) J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>6,0</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>4,4</td>
<td></td>
</tr>
<tr>
<td>Metal</td>
<td>30,0</td>
<td></td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>15,0</td>
<td></td>
</tr>
<tr>
<td>Protein-Water-Metal</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>Protein-Water-Protein</td>
<td>0,1</td>
<td></td>
</tr>
<tr>
<td>Calcium phosphate-Water-Metal</td>
<td>6,0</td>
<td></td>
</tr>
<tr>
<td>Calcium phosphate-Water-Protein</td>
<td>0,6</td>
<td></td>
</tr>
</tbody>
</table>

Data for the Hamaker constant and the hence the contribution of the van der Waals forces to the free energy of the system can be calculated from the dielectric constants (or refractive index) of the different components (Table 5). It is also possible to derive by contact angle measurements (Table 6). The benefit of using the surface tension is that the passivation of the surface by OH- groups on the surface can be accounted for.

The interfacial face tension or surface free energy attributed for the different forces can be estimated from contact angle measurements of the relevant surfaces [22]. The Young’s equation describe the correlation between surface tension, \( \gamma \) (SL solid/liquid, LG liquid/gas, SG solid/gas), and the contact angle \( \theta \)

\[
\gamma_{SL} + \gamma_{LG} \cos \theta = \gamma_{SG}
\]

The total interfacial tension (surface free energy) can, according to Visser [22], be estimated as the sum of the contribution from the van der Waal attraction and the Lewis acid base contribution.

\[
\gamma^{\text{tot}} = \gamma^{\text{LW}} + \gamma^{\text{AB}}
\]

Table 6 The surface tension (mJ/m²) calculated from contact angle measurements done on surfaces of importance for milk fouling studies.

<table>
<thead>
<tr>
<th>Surface</th>
<th>( \gamma^{\text{LW}} ) (mJ/m²)</th>
<th>( \gamma^{\text{AB}} ) (mJ/m²)</th>
<th>( \gamma^\circ ) (mJ/m²)</th>
<th>( \gamma ) (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium phosphate</td>
<td>43,3</td>
<td>0</td>
<td>0</td>
<td>5,80</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>40,8</td>
<td>0</td>
<td>0</td>
<td>3,17</td>
</tr>
<tr>
<td>( \beta )-lactoglobulin (20°C)</td>
<td>40,3</td>
<td>0</td>
<td>0</td>
<td>8,46</td>
</tr>
<tr>
<td>( \beta )-lactoglobulin (80°C)</td>
<td>37,9</td>
<td>0</td>
<td>0</td>
<td>7,73</td>
</tr>
</tbody>
</table>

Calculation of the contribution of van der Waals attractive interaction to Gibbs free energy for intermolecular interaction of \( \beta \)-lactoglobulin and its interaction with metal and calcium phosphate, gives negative values. The van der Waals contribution to the free energy is
expected to decrease with temperature but not enough to explain the fouling behavior of milk during heat treatment on its own.

### 3.2.2 Electrostatic repulsion forces

The Gibbs free energy for the electrostatic forces between the adsorbing particle and the metal plate at low (<50 mV) and constant surface potentials can be expressed as [22]

\[ \Delta G^{El} = 4\pi \varepsilon_0 \varepsilon_r \varphi_s \varphi_p e^{-\kappa L} \]

Where the \( \varphi \) is the surface potential for the sphere (s) and the plate (p) and \( \kappa \) is the inverse Debye screening length. \( L \) is the separation between the plate and the sphere and \( r \) the radius of the sphere. The so-called electrical double layer force when two charged surfaces are brought together with give rise to a repulsive force with a decay length, Debye screening length, of \( 1/\kappa \) (m) due the counter ions between the surfaces. The Debye screening length gives a measure of the width of the formed double layer:

\[ \frac{1}{\kappa} = \frac{\varepsilon_0 \varepsilon_r kT}{\sum_i (z_i e)^2 c_i} \]

Where \( \varepsilon_0 \varepsilon_r \) is the dielectric properties of the solution, \( k \) is the Boltzmann constant, \( z \) the valence and \( c \) the concentration of electrolytes in the bulk solution (ions/volume) [21].

The DLVO theory combines the attractive van der Waals forces and the repulsive electrostatic double layer forces originating from the charges of the two surfaces. If the electrostatic repulsion is significantly larger than the van der Waal attraction for protein particles, the energy barrier (significantly larger than \( kT \)) for the particle to attach to the surface will, according to the DLVO theory, be too high and no adsorption to the surface occurs. \( \beta \)-Lactoglobulin were measured to still form a monolayer on the surface, which means that other attractive forces than the van der Waals forces is needed to explain adsorption of proteins to stainless steel. Visser suggests that a Lewis acid/base interaction can be used to explain the phenomena [22]. For minerals like the calcium phosphate the attractive forces have been reported to dominate over the electrostatic double layer force and hence DLVO could explain the layer of minerals found on the steel surface [22].

### 3.2.3 Lewis acid-base and hydrophobic interactions

The concept of a Lewis acid base interaction is used by van Oss and Visser to model the additional force present causing the monolayer adsorption of \( \beta \)-Lactoglobulin that is not described by van der Waals forces or the electrostatic double layer [22, 23]. The Lewis acid base interaction is the polar interaction between an electron donor and an electron acceptor.

Calculating the Lewis acid base free energy, the energy for the interaction between calcium phosphate and a stainless steel surface, it is strongly negative at both high and low temperatures. This is also found for the formation of a protein layer on the surface when the
calcium phosphate is present. The calculated interaction energy is however positive for pure β-lactoglobulin in a solution without the presence of the salt component [22].

With estimation of the interactions influencing the Gibbs free energy of the initial step of fouling Visser suggests that both the electrostatic double layer and the Lewis acid base interaction can be used to describe the surface deposit [22]. It is also clear that the mineral content is important also for the formation of a monolayer of proteins.

3.3 The growth of a fouling layer

Fouling of heated surfaces has been an issue for the dairy industry since the start of pasteurization processes and therefor it has also been an important research field for quite some time. The general composition and structure of fouling have been related to the temperature of the heat treatment by classifying the fouling in distinguishable types [20].

Fouling at low temperatures, below the denaturation temperature of proteins occur on non-heated surfaces. The fouling present is mainly composed of a monolayer of protein and mineral deposit. Mineral deposition is faster than the adsorption of protein on to metal surfaces at low temperatures (below 55 °C) and the deposit has therefor a higher calcium phosphate content than protein in the non-tempered parts of the process system [24].

When heating the surfaces, the fouling increases. The buildup of the different fouling layers has been studied intensely. Quite a number of studies agree on that the process is driven by the unfolding and aggregation of proteins as well as protein interaction other milk components in the bulk. The interaction between colloidal particles and the surface is less important at this low temperature stage as they are few. Close to a heated surface on the other hand formed aggregates or particles will adsorb [15, 25, 26]. Unfolded and denatured proteins can adsorb due to hydrophobic interactions as a consequence of exposing hydrophobic residues during the unfolding processes leading to decreased solubility [27].

The precipitation of calcium phosphate increase due to dephosphorylation of casein at high temperature and the form calcium phosphate precipitate as the milk is heated. This lowers the pH during heat treatment together with the formation of acids in milk (Figure 9). The lowered pH causes hydrolysis of proteins which is of importance for the aggregation of milk proteins during heat treatment.

Caseins in milk are not sensitive to temperature as they lack secondary and tertiary structure and will therefore not unfold as the whey proteins with temperature. They will however aggregate when the pH change to close to close to the iso-electric point of the proteins. Furthermore they can react with the whey proteins as observed for κ-casein that will form disulfide bond with β-lactoglobulin. Denatured whey proteins will all react with the casein micelles and form aggregates if the pH is decreased below 6.5. Above 120 degrees casein molecules will also leave the micelle and to a larger extent react with the whey protein. This process is not only temperature dependent, but also observed at elevated pH (above 6.7) [7, 18].
β-lactoglobulin is the most abundant protein found in fouling at pasteurization temperatures, which in part can be explained by the denaturation temperature of the protein, which coincides with the pasteurization as well as the fact that the protein contains a free sulphhydryl group [28, 29]. Visser and Jeurnink have formulated a model of the adsorption and fouling from β-lactoglobulin (Figure 14). Native β-lactoglobulin protein exist as dimer under normal condition, but when the solution is heated the dimeric protein will dissociate into monomers and when the temperature reaches above 60°C the β-lactoglobulin will start to unfold and expose the free SH group [15, 30].

In the Visser and Jeurnink model aggregates or monomers of β-lactoglobulin can be bound to fouling layer, but also aggregate in the solution that can precipitate on to the surface and build up the fouling layer. At 80°C they suggested that aggregates that form on the surface are small and organized in a layered structure, whereas for higher temperatures larger aggregates are formed that are more difficult to close-pack. The fouling layer grows until the aggregates are so large that they are removed with the process flow. The formation of the larger aggregates with temperature, which cannot be close packing in fouling layer, and are more easily detached can be one of the reasons why aggregates of β-lactoglobulin and larger casein micelles are less common in the fouling layer at higher temperature [15].

![Diagram](image)

**Figure 9** The whey protein β-lactoglobulin adsorbs to surfaces as dimers even at room temperature and at normal milk pH, but will start to aggregate on the surface and form a thicker fouling layer when the temperature increase and in the presence of calcium ions. Casein micelles also react with the activated β-lactoglobulin aggregates forming the fouling layer. The salt components that are released upon heating and decreases in pH will either precipitate directly onto the stainless steel surface, creating a mineral deposit or binding to whey protein aggregates [15].

There are other studies by de Jong in the late 1990th and followed later by Grijspeerd in 2004 [26] that present somewhat similar ideas on how the fouling layer is built up. When the β-lactoglobulin starts to unfold, the molecules start to react in the solution together with other unfolded protein molecules. These aggregates are thought not to be included in the fouling buildup. The fouling is instead formed from unfolded protein molecules that react with other components of the milk [26].

Calcium salts that have been shown to be important for the fouling can precipitate in different forms onto the surface. Studies have been done on which complexes that are more likely to form, commonly by studying the ratio between calcium and phosphate in the fouling sample.
after ashing. As long as no irreversible complexes are formed, such as hydroxyapatite, Ca$_5$(PO$_4$)$_3$OH (Ca/P = 1.67), and the temperature is kept below 100°C, the precipitation is reversible. Hydroxyapatite is a crystalline form of calcium phosphate and the crystallization is usually disrupted by the interference by whey proteins and casein in the fouling layer [15].

The Ca/P ratio have been found to be close to 1.5 for the mineral content in type A fouling, which would match the amorphous complex tricalcium phosphate Ca$_3$H$_2$(PO$_4$)$_2$. For higher temperatures, causing type B fouling a lower ratio have been found, 1.2-1.35 [31]. Octacalcium phosphate, Ca$_8$H$_2$(PO$_4$)$_6$*5H$_2$O, with a ratio of 1.33 and brushite or dicalcium phosphate dehydrate (DCPD), CaH(PO$_4$)*2H$_2$O with the ratio of 1 are more crystalline than the tricalcium phosphate and could be part of the reason for the hard and brittle structure of type B fouling. Foster and Green found that the deposition at 140 °C has a layer enriched in mineral closest to the steel surface and that the fouling had a smoother surface than the fouling formed at 100 °C [32].

The processing before the main heating step influences the progress of fouling build up. One such example comes from the UHT treatment at 140 °C. The milk is most often pre-held at a certain temperature for a couple of minutes before entering the last heating section, to reduce the fouling build up (Figure 15). Without the preholding, type A fouling would form in a relative large amount and obstruct the flow (Figure 15, left) and increase the pressure drop in the system dramatically. The pre-holding step, prevent the type A fouling to form in the UHT treatment step. Instead type B fouling will start to form, with a linear increase in deposited amount as the temperature increases when entering the heater until the outlet (Figure 16, right).

Due to the build-up of the fouling layer, the thermal conductivity through the heat exchanger wall will decrease; therefore the temperature of the heating media has to be increased. This will cause the structure of the fouling layer next to the heated surface to change into a baked and more burnt fouling.

Carbohydrates are completely soluble in the aqueous phase of the milk at room temperature [6], but the sugar will react with free amino groups on protein residues during heating, via the so-called Maillard reaction (fig. 16). The amino group is in most cases from a lysine residue.
of a casein protein, and the loss of lysine after Maillard reaction will slightly reduce the nutritive values of the milk [16, 33]. The Maillard reaction is a complex chain of events that are not fully understood but as a final stage it will cause browning of the protein containing surface layer and also a change in taste and odor [16].

3.4 Conclusions regarding milk fouling

There are two main types of fouling found in the dairy processing equipment for heat treatment of milk, namely type A (<100 °C) and type B (>120°C). The protein content of the type A fouling produced below 100 °C is high, around 60%, but is drastically decreased on surfaces at higher temperature. The mineral content has the reverse temperature dependence compared to protein and the mineral concentration in the type B fouling is around 70%.

The crystalline structure of the calcium phosphate precipitating into the fouling at different temperatures differs. At lower temperatures the structure is amorphous, but the crystallinity increases with temperature. The precipitation that occurs below 100 °C has been observed to be reversible.

The colloidal forces discussed regarding the growth of a fouling layer are the combined forces of van der Waals attraction, electrostatic repulsion and hydrophobic interactions. They all seem to be important for the formation of the fouling layer. The mineral content is also observed to be of importance for the first layer of proteins to adsorb to the surface.
4 Evaluation of milk fouling

To be able to understand the mechanisms of the events during removal of fouling and the structural and compositional changes over time, good measuring techniques are of importance. The technique may be inline or off-line, depending on if the data need to be measured continuously or not. Not all techniques available are suited for industrial application, but mainly applicable for research.

4.1 Heat transfer changes due to dairy fouling

To understand the fundamentals of deposit removal from the heated surfaces, it is important to look into the process of heat transfer and the effect of the fouling layer. Characterization of the transfer of heat through a metallic material into a liquid is essential to the dairy industry.

Conductive heat transfer is in fluid a molecular transfer of energy, but the transfer heat through a stainless steel can be connected to the transfer of electrons in the material. Heat conduction is the way of transferring heat between the surface and the liquid at the stationary surface layer (at \( r=R, \) \( v=0 \)). Even for a turbulent flow, conductive heat transfer will be present due to the stagnant layer closest to the surface [34]. Convective energy transfer occurs thanks to the bulk motion of the fluid.

The transfer of heat and the temperature profile through the heat exchanger wall into the processed dairy product can be revealed by combining the heat transfer rate for the conductive heat transfer at the surface with the forced convective heat transfer from the surface into the bulk liquid. This can be done by applying Newton’s law of cooling.

\[
\text{Conductive energy flux vector: } \mathbf{q} = -k_i \mathbf{Nabla} T
\]

\[
\text{Newton's law of cooling } q = h(T_0 - T_b)
\]

\[
\text{Convective energy flux vector: } \rho \left( \bar{U} + \frac{1}{2} v^2 \right) \mathbf{v}
\]

Where \( k_i \) (W/m*K) is the thermal conductivity coefficient and \( h \) (W/m²*K) the convective heat transfer coefficient [34]. \( \rho \bar{U} \) is the internal energy and the \( 1/2 \rho v^2 \) is the kinetic energy per unit volume [35]. Combining the terms for conductive and convective energy transfer into the combined energy flux vector \( \mathbf{e} \) can be useful when calculating the heat transfer in a system. The convection is in this case is forced by an external force creating a fluid under flow.

From the perspective of the dairy industry and the heat processing of milk, it is possible to calculate the temperature needed for the surface to receive a certain outlet temperature, as long as the process equipment is clean. As soon as a fouling layer is formed on the heated surface the heat transferred to the bulk will decrease. To be able to correct for this change, the properties of the newly formed surface layer needs to be known. This is generally not the case for a fouling layer formed inside the dairy process equipment.
Evaluation of dairy fouling

Figure 11 Fouling during milk processing decreases the essential heat transfer to the product. The thickness, $L_2$, and a conductive heat transfer coefficient, $k_i$, for a fouling layer is normally unknown. The complexity increases due to the fact that both thickness and composition changes over time. The combined effect of $L_2/k_3$ is often referred to as the thermal resistance of the fouling layer. Idea for the picture adapted from [34].

The temperature differences over a composite material system (Figure 17) can be written as:

$$T_1 - T_2 = q \left( \frac{L_1}{k_1 A} \right)$$

$$T_2 - T_3 = q \left( \frac{L_2}{k_2 A} \right)$$

$$T_3 - T_4 = q \left( \frac{1}{h_3 A} \right)$$

The combined heat transfer rate can be calculated by adding the above equations and $q$ can then be expressed as,

$$q = \frac{T_1 - T_4}{\left( \frac{L_1}{k_1 A} + \frac{L_2}{k_2 A} + \frac{1}{h_3 A} \right)}$$

The overall heat transfer coefficient, $U$ (W/m$^2 \cdot$°K), can be introduced for the heat transfer through composite materials:

$$U \equiv \frac{q}{A \Delta T}$$

Giving the result for the heat transfer through the heated metal surface and the fouling layer:

$$U = \frac{1}{\left( \frac{L_1}{k_1} + \frac{L_2}{k_2} + \frac{1}{h_3} \right)}$$
By measuring the overall heat transfer coefficient relative to the value for the clean surface, $U_0$, the heat transfer coefficient for the fouling layer can be indirectly followed during both fouling and cleaning. This is in literature often referred to as the thermal resistance of the fouling, $R_F$ [36-43].

$$R_F = \frac{1}{U} - \frac{1}{U_0} = \frac{L_2}{k_2}$$

Calculating the corresponding value for deposit build up in a tube is slightly more complex, but following the same principle the generalized description of the overall heat transfer coefficient in the center of the tube, $U_0$, can be obtained:

$$\frac{1}{r_0 U_0} = \frac{1}{r_0 h_0} + \sum_{j=1}^{n} \frac{\ln \left( \frac{r_j}{r_{j-1}} \right)}{k_{j-1,j}} + \frac{1}{r_n h_n}$$

Where $L$ is now the length of the tube and $r$ is the thickness of the composite layer [35].

The temperature of the heated surface can, to a certain extent, be increased to compensate for the heat transfer due to the fouling build up. The extra heating from the surface will also cause baking of the deposit, which in turn will change the heat transfer properties, but also the cleanability of the surface after processing.

### 4.2 Pressure changes due to dairy fouling

Both the build up and removal of fouling from the tubular surface will cause a pressure change in the system, since the flow rate is constant but the inner diameter is changing. This pressure change can be used to monitor the system and have been used to model the behaviour of fouling [26].

As for heat transfer changes, measuring the changes in pressure to estimate the removal of fouling is an indirect measurement and does only produce relative values. The pressure drop in the pipes and tubular heat exchangers in the process equipment can be calculated with the Darcy’s law adapted for circular pipes.

$$\Delta p = \frac{f \rho v^2 \pi D^2}{2 \pi D \Delta L}$$

The friction factor, $f$, varies with the properties of the flow and also with properties of the pipe.

The Moody diagram (Figure 18) can be used to determine the friction factor. Laminar flow follow the Haagen-Poiseuille equation of mass rate of flow, $w$, over a cross-sectional tube area ($\pi R^2$)
Evaluation of dairy fouling

\[ w = \frac{\pi(P_0 - P_L)R^4 \rho}{8\mu L} \]

and the friction factor, \( f \), is found to be

\[ f = \frac{64}{Re} \]

The fully turbulent regime will not be dependent of the flow rate, only on the roughness of the pipe [44]. The relative roughness is determined by the absolute roughness and the hydraulic diameter \((\epsilon/D)\).

![Moody Diagram](image)

**Figure 12** Moody diagram showing the friction factor for pipes and it is dependence on flow rate and material roughness. Friction factor for laminar flow follow the Hagen-Poiseuille equation, whereas friction fraction for fully developed turbulent flow does not depend on the Reynold number.

For turbulent flow that are not fully developed, there are different ideas on how to explain the friction factor. Equations for smooth and for rough surfaces are usefull and has been combined into the Colbrook-White equation [44].

\[ \frac{1}{\sqrt{f}} = -2\log\left(\frac{\epsilon}{3.7D} + \frac{2.51}{Re\sqrt{f}}\right) \]

The pressure changes inside the system is commonly followed in the process industry and also for research purposes for following the buildup and removal of fouling online [20, 45].
4.3 Methodologies for measuring fouling buildup, composition and structure

There are several methods that can be used to study both the composition and the structure of the surface deposit. To characterize the composition, most methods are destructive and do not give any spatial information about the different elements.

The fouling layer closest to the product flow can be analyzed by several techniques such as ESCA and TOF-SIMS.

**Electron Spectroscopy for Chemical Analysis (ESCA)**

ESCA is also known as X-ray Photoelectron Spectroscopy (XPS) and is used for characterization of the chemical composition of the surface layer. The analysis can be done both in terms of qualitative analysis and as a quantitative analysis of the present atoms. The binding energy of two atoms can be calculated when the kinetic and the photon energy is known. The binding energy is specific for each element [46].

\[ E_k = E_b - h\theta \]

The result from ESCA is displayed as a graph with photoemission intensity as a function of binding energy (eV). The binding energy also depends on the neighboring atoms, which can be seen as a shift in the peak. The higher the electronegativity of the neighboring atom is, the higher the binding energy and the peak is shifted accordingly.

XPS has been used in order to determine the chemical composition of the fouling layer [47, 48]. The method is done under ultra-high vacuum and sample therefore needs to be completely dry, with the risk of collapsing the structure of the sample. The analyzed area is 500x500 µm with a penetration depth of 4-5 nm.

**Time of Flight-Secondary Ion Mass Spectrometry (TOF-SIMS)**

TOF-SIMS is a surface analysis method with higher sensitivity than ESCA and molecular composition and identification down to a depth of 1 nm from can be analyzed. Primary ions are used to sputter the first monolayer of the sample surface, producing emitted secondary ions from the material. The secondary ions are sent through the flight path, and are collected by a detector at the end. The detector is a mass spectrometer and a spectrum of the molecular composition on the surface can be generated. TOF-SIMS is to some extent a destructive analysis method, and the layers below the first monolayer are damaged by radiation and cannot be analyzed afterwards [11].
4.3.1 Content of organic compounds

The organic components of fouling include the different proteins, some unfolded, but also small amounts of lactose and citrate. There are several choices for protein determination; Kjeldahl method, UV absorbance or chemical oxygen demand (COD). Lactose and citrate are characterized by high performance liquid chromatography (HPLC).

Kjeldahl methods

The protein content in the fouling can indirectly been measured by the Kjeldahl method, which has been used since it was developed in 1883 [31, 49-51]. By using the method, the nitrogen content is determined by digesting the sample together with sulphuric acid and transforming it to ammonium sulfate [52]. Mercury, copper or selenium based compounds are used to catalyze the reaction.

\[
\text{organic C,} H, N + H_2SO_4(\text{boiling}) \rightarrow NH_4^+ + CO_2 + H_2O
\]

The solution now containing the free \(NH_4^+\) is treated with an alkali solution, forming gaseous ammonia. The ammonia is distilled into a container of HCl and the final amount of nitrogen in the sample is determined by titration of the HCl that has not reacted with the ammonia [53].

UV absorbance

By measuring the absorption of ultra violet light at one or two wave-lengths, the concentration (g/L) of protein in a solution can be determined. The absorption is dependent of the pH and lipid/protein ratio. As long as the lipid/protein ratio is below the critical 0.05, an equation for the calculation of protein content at pH 13 can be used without overestimating the protein due to the scattering from lipid aggregates. For the two wave length used, the mineral concentration will not interfere in the measurements [54].

\[
\text{protein (g/L)} = \frac{(A_{248} - A_{256})}{\overline{A}_{248} - \overline{A}_{256}}
\]

Where the measure of \(\overline{A}_{248}\) and \(\overline{A}_{256}\) originates from measurements on a standard protein solution. Xin et al used the UV absorption and used a similar equation for the use of dissolved whey protein concentrate (WPC) [55].

\[
\text{WPC (g/L)} = 1.0368(A_{248} - A_{256}) + 0.0005
\]

The measurements performed were done continuously during the cleaning and was used to calculate the cleaning rate of the model fouling. The sensitivity of the absorption method is 1 mg/L.
Evaluation of dairy fouling

Chemical oxygen demand (COD)
COD is a method to measure how much organic compounds that are available in a liquid solution. It is measured as the amount of oxygen (mg/L) that is needed to oxidize the organic compound to carbon dioxide, ammonia and water. This analysis has been used in several studies for analysis of effluent liquid after cleaning of protein fouling [5, 56].

High performance liquid chromatography (HPLC)
High performance liquid chromatography is a separation either by size, charge or by affinity. The sample of interest is in a solution that is passed through a column specially designed for the molecules of interest. The result is shown as elution peaks with Gaussian spread and the resolution between to peaks is given by

\[
Resolution = \frac{\Delta t_r}{w_{av}}
\]

The retention time, \( t_r \), from the point where the liquid is injected to when it is again eluted after the column into the detector cell, and is specific for the elements of separation. The average spread of the two peaks is denoted \( w_{av} \) [53]. In cleaning investigations the method has been used to measure the content of lactose and citrate in the effluent liquid [56].

4.3.2 Mineral content
When the mineral content of the fouling is studied, the organic content is most often first removed. This is often done by ashing the sample, leaving the inorganic material. This will give the total mineral content in the sample. For further analysis the different atomic adsorption spectroscopic methods are used.

Atomic absorption spectroscopy (AAS)
Atomic absorption spectroscopy can be used to determine the concentration of a specific element in sample and measures the light absorbed by free atoms in the sample. The sample to be analyzed is evaporated in a gas flame at a temperature between 2000 - 3000 K.

For the absorption spectroscopy an iron cathode is used that are exited and vaporized when in contact with the gaseous flame. The ferrous atoms will emit light that is detected on the opposite side of the flame. The sample atoms burning in the flame will absorb energy from the light emitted by the ferrous atoms and the intensity loss will be detected as peaks on the spectra. By using a graphite furnace instead of a gas flame solid sample could be directly analyzed instead of dissolving them in a liquid [53].

There are other spectroscopic methods, one of which is the emission spectroscopy (flame photometry). The different methods have different limits when it comes to sensitivity (Figure 19) and the sample volume needed. The atomic absorption has been used by several researchers studying both build up and cleaning of fouling from dairy products [31, 49, 56].
Flame photometry
Flame photometry is a kind of a simple atomic emission spectroscopy, but can also be used as a detector connected to for example a gas chromatograph. For atomic emission spectroscopy there is no need for an external light source. The molecules are burnt and separated by the gas flame, exiting the elemental atoms. The wavelength of light that are emitted when the electrons go back from the exited state to the ground state are specific for each element and the intensity of the light can most often be translated proportionally into the quantity of the atom[53]. Calcium ions is measured at 622 nm [14, 57] and phosphorous have an emission at 536 nm [53].

4.3.3 Structural analysis

Structural analysis can be done in several ways, depending on the information of interest. With methods like scanning electron microscopy (SEM) or atomic force microscopy (AFM), the knowledge gained are generally on the topography, whereas a three dimensional image of the sample can be obtained from the use of transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). By the use of CLSM it is also possible to separate different components from each other, provided they can be labeled with fluorescent probes.

Scanning electron microscopy (SEM)

Scanning electron microscope (SEM) is a microscope that detects electrons that are scattered or emitted from a specimen surface due to irradiations of the surface with an electron beam. With this technique, it is possible to get topography images of the surface of a sample. The surface investigation is often performed in vacuum environment and one protruding challenge with the analysis of soft milk fouling, which normally contains a lot of liquid, is to preserve the structure when preparing the sample.

One additional possibility is to use an environmental SEM, which allows certain humidity. The resolution of such a method is less than the vacuum SEM due to scattering of electrons by the water present. A qualitative analysis of the surface composition can be performed if energy-dispersive X-ray spectroscopy (EDX) is used together with the SEM.

Transmission electron microscopy (TEM)

The transmission electron microscope forms a major analysis method in a range of scientific fields, in both physical and biological sciences. The transmission electron microscopy can help to find out about the size, shape, and arrangement of particles on a surface, but also allowing identification of the chemistry of the sample. One of the major drawbacks of TEM is that it provides 2D images out of 3D samples, making result interpretation a challenge. Sequence of images is able to create a 3D image. However, development of imaging techniques, where the sample is tilted has made it possible to construct 3D images of the sample. Many materials also require extensive sample preparation to produce a sample thin
enough to be electron transparent, which makes TEM analysis a relatively time consuming process.

**Atomic Force Microscopy (AFM)**

Atomic force microscopy (AFM) is another method to study the surface topography on a nanometer scale. It is challenging to study milk fouling with AFM the surface is heterogeneous and soft. The technique is sensitivity to large differences in depth of the surface. However, one advantage is that it can be used to reveal the rheological properties of the surface layer.

**Confocal laser scanning microscopy (CLSM)**

The structure and topography of the fouling layer could be useful to follow over time. Information to be gained from this analysis could be to visualize diffusion and back diffusion through the fouling layer. One could also get an idea whether the fouling is shredded off in layers or rather removed in bigger pieces.

Confocal microscopy register a 2D image of a very thin section of the samples at a certain depth and was first suggested by Marvin Minsky in 1955 [58]. By recording images at varying depth and stacking these images on top of each other it is possible to create a 3D image of for example a cross section of the object [59]. The method uses either light reflected from the specimen or light released by excited fluorophores.

CLSM is most widely used in the field of microbiology [60] and is often used to study thin section of biofilms [59, 61, 62]. But it has been used to study fouling layers from heating of milk. CLSM has for example been used together with fluid dynamic gauging for the analysis of a gel swelling process during cleaning in an alkali solution [63]. Since the confocal microscope is still using light as a source the magnification is relatively low in comparison with for example a microscope that uses electrons as source of energy.

**4.3.4 Thin film adsorption and desorption**

The initial adsorption and deposition to a large extent determines the buildup of fouling layer and it is therefore important to understand the detailed mechanisms involved in this initial process. Quartz crystal microbalance and ellipsometry are methods known for their use in this purpose.

**Quartz crystal microbalance with dissipation (QCM-D)**

QCM-D is a technique used to in situ measure adsorption on to a surface. The QCM-D is simplified, a very sensitive scale that measures the weight of a sample that attaches to the sensing crystal surface. Part of the popularity of QCM-D is due to the fact that no labelling of the samples is needed and is still a very sensitive method.

The QCM-D instrument is a quartz crystal to which a layer of gold is deposited, serving as electrodes. The crystal material is piezoelectric and can be brought to oscillate at a specific
resonance frequency when an oscillating electric field is applied. The resonance frequency is dependent on the mass of the crystal, hence the resonance frequency will change with $\Delta f$, when the sample adsorbs to the surface [64].

As long as the adsorbed mass is evenly distributed, rigidly attached, and small compared to the mass of the crystal, the Sauerbrey equation can be used to evaluate the adsorption:

$$\Delta m = -\frac{C\Delta f}{n}$$

C is a constant (0.177 mg•m$^{-2}$•Hz$^{-1}$) and n is the overtone number.

**Ellipsometry**

Light reflection can be used to study the thickness of a layer and has been done so since the days of Sir Isaac Newton. Ellipsometry is widely used to study thicknesses of thin films, below the wavelength of visible light, using the changes in polarization state that occurs when (polarized) light is reflected against the sample surface [65-67]. Depending on the optical contrast and the reflectivity of the sample, the thickness resolution of this technique can be 0.1-1 nm [68].

From the values of refractive index and thickness the amount adsorbed can be calculated, provided that the refractive index as a function of concentration (dn/dc$\_i$) of the adsorbing specie is known.
5 Model fouling for studying fouling build up and cleaning processes

Fouling and cleaning are complex processes and to gain fundamental understanding of the mechanisms it is sometimes convenient to begin the investigation with a fouling liquid less complex than the actual processed liquid. The question is then which model fouling to use in order to gain fundamental and detailed knowledge about mechanisms, but still to be able to study relevant properties. During the decades of research within the field of milk fouling and cleaning, one of the difficulties has been to find such a model system, which also is reproducible and easy to obtain.

5.1 Production of model fouling

The method used to create the model fouling will influence the properties. Passing a heated solution over a stainless steel plate, preferably heated, will cause fouling to form on the surface in a manner similar to the one occurring in the process equipment. The concentration of protein solution used to build up the model fouling is often selected to mimic the concentrations in milk, specifically the concentration of β-lactoglobulin (3 g/L) [67, 69]. The deposition of the protein and in some cases a combination of protein and minerals relevant for products to be heat treated is fairly common as means to prepare a model fouling.

Another way to form model fouling is to create a gel from the whey protein. Heat induced gels are produced by heating the solution to the denaturation temperature of the proteins; this will cause proteins to aggregate to form a gel [70-76]. Heat induced gels are one of the most common model fouling to use when studying the dissolution behavior during alkali treatment (Table 7). Gel formation is pH dependent and hence gels can also be formed by altering the pH of the protein solution before heating [70, 75]. Many globular proteins will form particulate gels, seen as opaque, when the pH is around the isoelectric point for the protein (pH 5.4 for β-lactoglobulin), and stranded transparent gels at low and high pH.

Solvent evaporation from milk on a surface creates a thin film, but such a film will not have a structure similar to the real fouling since it collapses when the liquid is removed. Studies of the mineral fouling are also done mostly by evaporating the solvent from either a simulated milk ultra filtrate (SMUF) solution or a calcium phosphate solution on the sample plate. SMUF is a combined solution of the minerals found in milk. The crystallization of mineral is influenced by the presence of proteins during the fouling process, but the protein deposition is also influenced by the mineral content. This information is generally lost using a simple model fouling.
Model fouling

Table 7 The variety of model fouling is vast and this is important to consider for the interpretation of the results of any study. Protein fouling could be relevant for mimicking type A fouling, whereas studies of the type B fouling needs the input of a mineral deposit.

<table>
<thead>
<tr>
<th>Model fouling</th>
<th>Temperature (°C)</th>
<th>Method</th>
<th>Previous work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein concentrate (WPC)</td>
<td>&lt;100</td>
<td>Circulation through a heated section or forming gels</td>
<td>[39, 70, 71, 77-84]</td>
</tr>
<tr>
<td></td>
<td>&gt;100</td>
<td>Circulation through a heat exchanger</td>
<td>[43, 45, 50]</td>
</tr>
<tr>
<td>Whey protein isolate</td>
<td>&lt;100</td>
<td>Gel formation</td>
<td>[47, 49, 70, 85, 86]</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>&lt;100</td>
<td>Heated flow cell, pH 6.8</td>
<td>[82, 87]</td>
</tr>
<tr>
<td>Protein + SMUF</td>
<td>&lt;100</td>
<td>Evaporation</td>
<td>[42, 69, 89-91]</td>
</tr>
<tr>
<td>SMUF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk (both full and skimmed)</td>
<td>&lt;100</td>
<td>Evaporation</td>
<td>[19, 28, 29, 56, 92-95]</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>&lt;100</td>
<td>Infrared drying</td>
<td>[91]</td>
</tr>
</tbody>
</table>

5.2 Model fouling to mimic type A fouling

Several different milk proteins and protein mixtures are used today, making comparison between results complicated. Common is the use of a model based on protein, either from whey protein isolate (WPI) or whey protein concentrate (WPC). WPI is a more pure protein powder than the WPC and contain less minerals and lactose (Table 8). For studies of milk fouling representing type A fouling, mostly containing protein, this kind of system is often used. The most abundant protein in the milk fouling at these temperatures is β-lactoglobulin and a few research groups have used the pure protein to form a gel film on a surface [72-74, 76, 87].

Table 8 Composition in wt.% of model system fouling used in literature [70].

<table>
<thead>
<tr>
<th></th>
<th>β-lactoglobulin</th>
<th>WPI</th>
<th>WPC80</th>
<th>WPC35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>97.4</td>
<td>90–94</td>
<td>80.1–83</td>
<td>34-35</td>
</tr>
<tr>
<td>Fat</td>
<td>0.1</td>
<td>&lt;1</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.8</td>
<td>4.3</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt;0.5</td>
<td>&lt;3</td>
<td>6.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Ash</td>
<td>2.4</td>
<td>2.0</td>
<td>3.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

5.3 Model fouling to mimic type B fouling

To produce the type B fouling temperature above 120 °C is needed and also a high concentration of minerals. UHT treatment of only a WPC solution will mainly produce type A fouling [45]. For studies on the formation and properties of the type B fouling formed during UHT treatment of milk, most researchers have so far chosen to study the real fouling and not used a model system [20, 31, 32, 96].
6 The importance of cleaning - to obtain and evaluate the clean surface

A customer expects a high and even quality of the product. The traceability is therefore an issue in the dairy industry and every step in the process needs to pass the requirement for hygienic handling of food. Every batch is tested before leaving the dairy both for immediate contaminations, but also for long time preservation studies. The most common analyses are cultivation for bacteria and mold, but also sensory tests of the product such as texture, smell and taste.

The heat treatment used in the dairy industry causes fouling and thereby imposes difficulties with heat transfer of the milk and but can also cause off-flavors. The produced fouling needs to be removed to ensure a safe process. Parameters discussed as relevant for cleaning is the temperature of the cleaning solution as well as the concentration of cleaning agent, the time and the flow parameters. These four parameters, used when discussing cleaning-in-place (CIP) are usually visualized in something called the Zinner’s circle (Figure 20). Note that the effects of pH dependence and mechanical shear forces are also included in the parameters of flow and detergent concentration.

![Figure 13](image)

Figure 13 The Zinner’s circle visualizes the four parameters that are usually considered of importance for the cleaning efficiency of a CIP process. The internal importance of the four parameters is important for the optimization of the process.

6.1 Cleaning of different fouling types in the dairy industry

Most dairy equipment for processing milk and milk products is cleaned with a so called CIP system. CIP or cleaning-in-place means that the equipment is cleaned without dismantling. Such a program is designed to make certain that the equipment is clean without the need to open and look at the single parts of the system. An insufficient cleaning creates an environment where microorganisms can survive and multiply. Left over fouling will also act as a perfect nucleation site for the new fouling from the next batch of production, increasing the fouling behavior of the heat-treated product.
Since there are different ways to process milk products, there are also different ways in which to clean the equipment, depending on the presence and severity of deposit on the surfaces. With a fouling consisting of protein and fat, an alkali treatment is a sufficient method. There are no industrially produced milk fouling that consists of only protein and fat and the acid cleaning step is therefore always of importance. Acid detergents are used in the cleaning process in addition to the cleaning with alkali in order to remove the inorganic part of fouling from the surface. The acid step also decreases the possibility of microbial growth if they are not efficiently washed out [97].

Heated and non-heated surfaces do not necessarily need the same cleaning program. The non-heated surfaces could be cleaned with alkali at a low concentration, even though the alkali step traditionally is followed by an acid step. The heated equipment from pasteurization process (with a buildup of type A fouling) a higher concentration of alkali is used [98, 99]. For products processed at temperatures above 110 degrees, for example the UHT-treatment, the fouling is a more complex mineral rich fouling and is cleaned with an alkali and acid detergents at higher concentration than for pasteurization [99, 100].

Cleaning procedures in the food industry often follow the same patterns and does often include the steps of pre-rinsing, detergent cleaning, intermediate rinsing if more than one detergent step is needed and a rinsing step at the end to flush the system with water before the following production [101]. The type of detergent used in the cleaning process differs, where the equipment producers often recommend a detergent concentration based on pure alkali and acid chemicals such as NaOH and HNO₃, while others prefer formulated solutions.

The formulated detergents are supplemented with sequestering or chelating agents binding either one or several of the mineral ions attached to the fouling. Surfactants can also be added in order to disperse the fat into the solution. Surfactant and emulsion behavior is often temperature dependent and the formulated cleaning solution often have optimal temperature interval (usually 40-90°C) [102, 103].

It is possible to use a one-stage or a two-stage cleaning process of fouling from dairy products, depending on the present fouling. One stage cleaning could be an alkali cleaning of non-heated surfaces or the use of a formulated alkali detergent on a lightly fouled surface. Two stage cleaning processes contain one alkali and one acid cleaning stage [40, 84]. Heavily fouled surfaces, such as the heated surfaces forming both type A and type B fouling, uses combination of the formulated alkali solution as for the one-stage cleaning, but with the added acidic cleaning step.
7 To understand the cleaning process

Designing an optimal cleaning process is difficult without the knowledge of the deposit structure, composition and mechanisms involved during cleaning. The fouling produced during heat treatment of milk is heterogeneous and varies throughout the different sections of the processing equipment. The cleaning process has to be designed to take into account the previous process of heat treatment of the fouling liquid and the duration of production. These issues makes milk a complex liquid to study, and the milk fouling has been classified as type A and B in order to characterize the mechanisms of fouling removal during cleaning.

Studies of protein removal, mimicking type A fouling from pasteurization temperature, has been frequently studied and several models for the removal mechanisms can be found [56, 80, 84, 104, 105]. For protein removal, diffusion of cleaning solution is necessary and the swelling of protein layers due to alkali solution, as well as other chemical reactions occurring during buildup of the fouling layer. The impact of mechanical force on the removal by the flow has been questioned [106].

The study of removal of type B fouling is more complex and more expensive, since both the production and cleaning is performed at such temperatures that pressurized equipment is needed. The initial challenges involve producing a fouling representing the one found after high temperature treatments. The few studies of high temperature fouling (type B) have been done on larger scale, either processing thousands of liters of milk or recirculating the liquid that affects the properties of the fouling created [5, 26, 31, 107]. Solubility and dissolution of pure mineral deposit from a stainless steel surface has also been studied. This study conceive that both dissolution and removal due to shear forces from the flow is of importance for the mineral deposit [91].

A cleaning model that is possible to adapt from a type A to a type B fouling has so far not been presented. The mechanism for cleaning of the predominant mineral based type B fouling cannot solely be explained by evaluating and understanding the cleaning of a mineral or a protein layer, but a combination of the two types of deposits has to be considered. This chapter will first introduce the cleaning mechanisms evaluated previously to understand and explain the phenomena acting during the cleaning process, and then explain the models and calculations made that support the experimental findings and the theoretical hypothesizes.

7.1 Mechanisms involved in the cleaning process

Since most studies are performed on the protein rich type A fouling and on model fouling based on proteins, the mechanisms in focus are the reactions occurring between the cleaning agent and the protein layer structure. The mineral deposit available in the type A fouling is thought to either be transferred into the bulk together with the protein content [56] or through a dissolution process. For the Type B fouling from high temperature processing, the
understanding of mineral removal is essential. Studies on pure mineral fouling indicate that minerals are essentially removed by dissolution in acidic environment [91].

The diffusion of the cleaning agent is of great importance at the beginning of the cleaning process. Without the first diffusion the chemical and physical reactions between fouling and the cleaning agent will not be possible, except from the outmost surface layer. Most researchers do not consider the diffusive motion to be a rate limiting step in the process but still an absolute necessity for the cleaning to be efficient [71, 84].

Diffusion can be described as the net flow of cleaning agent due to a concentration gradient into the fouling layer [21] in through the stagnant boundary layer.

$$J_i(y) = -D_i \frac{dc_i(y)}{dy}$$

Diff is the diffusion coefficient and can either be determined experimentally or estimated based on the size of the molecule. The diffusion coefficient for the hydroxyl ion present during alkali cleaning has been approximated to sphere with the Stoke-Einstein approximation.

$$D_i = \frac{kT}{6\pi\eta R}$$

Where R is the radius ($R_{OH} = 1.3*10^{-10}$ m), \(\eta\) is the solution viscosity (Ns/m$^2$) and k is the Boltzmann constant (1.38 *10$^{-23}$ Nm/K). Using the Stoke-Einstein approximation, $D_{OH}$ in a 20 °C diluted water solution would theoretically be approximately 1.65 μm$^2$/s. Jeurnink and Brinkman claims that the effective diffusion coefficient is even slower and in the range of 10$^{-8}$ m$^2$/s [56]. Diffusion of dissolved compounds through the fouling and the boundary layer is also present in the opposite direction while removing the fouling from the surface. Molecules and small particles detached from the fouling layer will transfer into the bulk due to the concentration gradient.

Swelling of protein fouling during cleaning with alkali, such as NaOH, has been monitored and is part of most cleaning models for protein systems [72, 83, 84, 105, 108]. Swelling can be visualized as a combination of chemical and physical reaction. The hydrolysis of the protein tertiary structure is partially due to α and β elimination of the disulfide bridges. This is only possible for proteins that contain the amino acid cysteine, which contain sulfur and are able to form strong disulfide bridges. β-Lactoglobulin, which is the predominant whey protein in type A fouling deposit [31], can readily form disulfide bridges and can thus be collapsed by the reaction with hydroxyl ions, causing swelling of the protein layer.

Inside a protein-based fouling layer a range of reactions are likely to occur, when the cleaning agent molecules enter the network. The heat used to coagulate and precipitate proteins has altered the internal structure of the protein and exposed internal hydrophobic amino acid residues. The unfolded protein can react with hydroxyl ions entering the protein layer, which can lead to (partial) hydrolyse of the protein and disentanglement of the protein network. The (negative) charge density is likely to be increased as the increase in pH, which in turn leads to
an increases intra- and intermolecular electrostatic repulsion that causes swelling of the fouling layer.

![Figure 14](image_url) Swelling of protein gels occur even at low concentration of alkali. WPC gels were submerged in alkali solutions at 20 °C with different NaOH concentration (0.1, 0.5, 1.0 %, which should correspond to a pH of 12.4, 13.0 and 13.4). The swelling is largest at 1% NaOH, that swelled almost with 100 % of the initial volume within 10 minutes [80].

Alkali solutions will cause protein fouling to swell even at low concentrations (Figure 21). When using NaOH to remove a protein deposit, an optimum concentration has been found at 0.5-1% (w/w) or 0.1 M [76]. A higher concentration can create a gel layer on top of the fouling, which restricts the transport of particles detached from the fouling layer into the bulk and thus hampers the cleaning process [56, 76]. This phenomenon has been seen in lab scale and for model fouling, but has so far not been observed in the industry where the chemical concentration is generally larger than 1% and the temperature is also higher.

The main aim for using an acidic cleaning agent is to dissolve the mineral deposits by lowering the pH. Calcium phosphates have a decreased solubility with increasing temperature and can therefore not be dissolved by the warm regular cleaning solution, but needs the pH to be lowered in order to disrupt the balance in the crystal formation.

\[ 2H^+ + Ca_3(PO_4)_2 \rightarrow 2H(PO_4)^{2-} + 3Ca^{2+} \]

The acidic cleaning agent is consumed in the cleaning process, which needs to be taken into account for in the dosing. The regular concentration for acidic cleaning agent in the dairy industry is in the range of 2% for heated and heavily fouled surface.

The fouling layer removal are not only determined by the individual components but can be attributed to the properties of the whole of the fouling layer. Such properties are the cohesive and adhesive strength of the layer. Cohesion is the forces holding the internal structure whereas the adhesion is the force keeping the structure to a foreign material, in this case the stainless steel of the process equipment.

The adhesive and cohesive properties of the fouling has been found important, but there is not a straightforward answer to which one is the strongest in a protein fouling layer [56, 80]. Micromanipulation shows differences in these properties between for example different food fouling. Tomato paste has higher cohesive properties whereas milk protein (WPC) fouling has
shown a higher adhesive strength (figure 22) [80]. The adhesive forces in milk protein fouling is affected by the protein the properties and differ between native and denatured globular protein [27].

![Figure 15](image)

**Figure 15** Milk protein fouling produced on a metal surface at 90°C has a higher adhesive strength than the cohesive fouling strength further from the steel surface. The graph show the energy needed to pull a probe through the sample, and has been shown to differ between different fouling products [80, 81].

Whether the adhesive or cohesive properties dominate is important for the way the fouling layer is removed, i.e. if shredding of protein in layers and ripping lumps of protein from the surface occurs [84]. The transfer mechanism of fouling components into the bulk cleaning solution was discussed in a paper from Christian and Fryer from 2004, where they show that both cohesive and adhesive forces might be available and that there is a possibility to change the mass transfer mode of proteins by changing the alkali concentration [43].

Different strategies have been tried to modify the stainless steel in order to develop a new non-adhesive surface for processing liquids that are known to cause severe fouling. Changing the surface energy and/or topography, changes the adhesive properties, which in turn can affect both the formation of the fouling layer and the cleanability of the surface [38] [48].

The mechanical forces are not static and will change both locally at each time point, but also over time due to the changes in fouling properties. Cleaning is done in a fully developed turbulent flow (Re >2300), but the irregularities in fouling might contribute further to increase the turbulence. The flow will transport loose substances from the surface quickly, creating the concentration gradient for new molecules to be transported out from the fouling layer.

Shear is the result of the difference in velocity between the fluid motion and the stainless steel surface and will be influenced by turbulent motion. Shear forces are therefore of course important for removal rate of fouling. Mineral fouling is considered to be smooth [91], not creating increased turbulence in the flow pattern.
7.2 Models to describe the cleaning process

The mechanisms involved are highly intertwined and changes over time as the fouling structure and composition changes. In order to bring clarity to the problem, descriptive and mathematical models have been developed, each simplifying the problem to produce a solution that is applicable to the situation. Most models produced so far does not consider the whole complexity of the milk fouling, but focusing on either the protein or the mineral part of the fouling.

The protein-based models can represent the type A fouling that has a high concentration of protein. The removal is then often regarded as a step-by-step reaction producing a perfectly layered fouling/reaction front, with clearly specified steps. A protein system is not appropriate to use to draw conclusions on the cleaning process of type B fouling, since a more complex model system including proteins a high mineral concentration has to be used. For model systems based on different content of the deposit, a direct comparison of the models is difficult. Cleaning models that does not directly correspond to reality can still cast some light over the many questions that so far have been un-answered. However, the benefit of using a combined protein and mineral fouling in the search for a solution of the issue of cleaning is clear.

The models introduced in this chapter are based on different fouling systems, which is important to keep in mind when comparing models. The models presented are well known in the field and frequently referred to when looking into the cleaning process of milk fouling.

7.2.1 Gallot-Lavalleé and Lalande 1985

The work presented by Gallot-Lavalleé and Lalande in 1985, were done on a heterogeneous fouling. The fouling used was created in a pilot pasteurization of 2000 L raw milk, creating a fouling corresponding to the type A group. The cleaning agent used was NaOH with concentration varying from 0.1 to 3.9 %. The model is based on the data of online measurements with optical sensors measuring turbidity changes [96].

![Diagram of cleaning process](image)

**Figure 16** According to the model suggested by Gallot-Lavalleé and Lalande, the mechanisms involved in cleaning is the diffusion of sodium hydroxyl ions through the fouling layer. The protein layer swells, forming an intermediate layer, Y. Units of protein fouling will detach from the fouling, and will be transferred first by diffusion through the protein layer Y and then by the fluid motion. The diffusion of Y into the formation of Z is considered to be the rate limiting step [96, 105].
Based on studies of the fouling layer during the cleaning process, Gallot-Lavalleé and Lalande suggest that the cleaning agent changes the fouling to layered structure (Figure 23). Both the transformation of the unreacted fouling, \( X \), into the swelled intermediate fouling, \( Y \), and the final transformation of \( Y \) into removable units, \( Z \), is thought of as first order reactions [96, 105]. The reaction rate constants for the two stages seem to be equal giving the reaction rate for the formation of the final units, \( Z \), to be:

\[
r_Z = M_0 k t e^{-kt}
\]

\( M_0 \) is the initial mass of the fouling. The reaction rate \( r_z \) was seen first to increase, reach a maximum and then decrease again. This suggests that the limiting step of the cleaning is the reaction where the swelled protein detach and form the final units, \( Z \) [105].

The reaction stages have been described mathematically, using first order reactions. The first stage in the cleaning process is the external transfer of hydroxyl ions to the fouling surface followed by the internal diffusion of hydroxyl ions inside the fouling. The rate is calculated by using Fick’s law of mass flux:

\[
r_{OH,1} = (c_{OH,bulk} - c_{OH,surface}) \beta_{OH}
\]

\[
r_{OH,2} = (c_{OH,surface} - c_{OH,Y}) \frac{A \rho_Y D_{OH}}{M_Y}
\]

\( \beta_{OH} \) is the mass transfer coefficient of hydroxyl ions between the bulk and deposit surface. Rate of internal diffusion is dependent on the area, density of the intermediate fouling \( Y \) and mass of swelled fouling \( M_Y \). The diffusion coefficient \( D_{OH} \) can be calculated from the Stoke-Einstein relation for a spherical particle.

\[
D_{OH} = \frac{kT}{6\pi \eta_{OH} R_{OH}}
\]

The expression for the diffusion coefficient \( D_{OH} \), clearly indicates that the increase in the viscosity due to swelling of the protein layer will induce a lowering of the rate of diffusion through the fouling.

The reaction, where the original fouling come in contact with the cleaning agent, which make it swell to form an intermediate fouling layer occurs in the reaction zone \( f \). This can described as a first order reaction depending on the concentration of hydroxyl ions and the thickness (\( F \)) and the number, \( n \), of \( X \) reacting with the cleaning agent.

\[
-r_X = nF k_x c_{OH,Y}
\]

The temperature dependence according to the Arrhenius law of the reaction rate of fouling \( Y \) swelling of the can be expressed as

\[
k_x = k_0 e^{(\frac{1}{T_0} - \frac{1}{T}) E_r / R}
\]
Where $E_r$ is the activation energy (kcal/mol) and $R$ is the ideal gas constant. The mass transfer of loose intermediate fouling in the swelled layer is described as

$$-r_Y = \frac{M_Y \beta_D}{V} - n f F c_{OH,Y}$$

$\beta_D$ is the mass transfer coefficient of the protein residues released from the network structure. $M_Y$ is the mass of fouling residues from the swelled volume, and $V$ is the total volume of the tube. The formation rate of the final units that will be transferred from the fouling layer to the bulk liquid is described in a similar way

$$r_z = \frac{M_Y \beta_D}{V}$$

Similar models with a reaction front or reaction zone have also been published by Bird and Fryer, 1991 [30], but also Xin et al in 2004 [84]. These models will be discussed below.

### 7.2.2 Bird and Fryer 1991

In 1991 Bird and Fryer was published a cleaning study based on a model fouling made by pumping a 3% whey protein solution through a pipe at $Re = 6000$ at a temperature from 75 to 95 during one hour [30].

The authors investigated cleaning as a function of temperature, flow and cleaning agent concentration, referring to the work done in the late 50th by Jennings and coworkers. These are also the same parameters varied by Gallot-Lavalleé et al [96, 105]. The cleaning agent used was also the sodium hydroxide.

The cleaning rate followed the same pattern as seen in other studies where there is a rapid increase in the beginning, which then levels out and then decreases. This observation has been done by several other researchers afterwards [84, 106], although for the Bird and Fryer study it is shown that the temperature needs to be above 60°C to have an significant increase in cleaning rate. The change in cleaning rate between 50 and 60 °C (Figure 17, left) was found to be so large that it could not only be an effect of the increase of diffusion rates and dissolution that occur at the elevated temperature [30]. This increase in cleaning rate occurs at about 60 °C, which also was reported by Gallot-Lavalleé and Lalande [105]. The activation energy barrier for some chemical reaction inside the fouling layer, which is important for the cleaning process, seems to have been reached at this temperature.
Figure 17 Left graph shows the removal rate at different temperature at a cleaning agent concentration 1% NaOH. Right graph shows the effect of cleaning agent concentration at 50 °C is seen [30]. Color lines are added to highlight the measured difference between 0.5% and the 2% NaOH often seen for heated surfaces in the dairy industry.

At low temperatures like 50 °C a clear initial increase in cleaning rate with cleaning time (Figure 17, right) is seen even for alkali cleaning solution concentrations far below used in the industry.

Based on imaging data of the cleaning process with SEM, Bird and Fryer suggests that the removal of the fouling is not uniform and is removed in lumps. But they also observed that fouling treated with high concentration of NaOH will form a translucent protein layer that only disappears very slowly, indicating that for this type of fouling exist an optimal alkali concentration. The formation of a smooth gel like layer due to high alkali concentration is also discussed by Jeurnink and Brinkman, 1999, and Mercadé-Prieto et al 2008 [56, 76].

7.2.3 Jeurnink and Brinkman 1994

In 1994 a model of the mechanism for removal of milk fouling produced by pasteurization of milk was presented (Figure 26) [56]. The fouling is removed with both an alkali and an acid cleaning step. By monitoring the removal of both protein and mineral deposit a model of the fouling layer removal, based on the transfer of the fouling as larger pieces from the surface to the bulk, was developed. The fouling layer here is considered divided into a voluminous protein layer on top of a thin and dense mineral layer next to the surface.
The alkali cleaning solution is thought to diffuse into the protein fouling layer and cause chemicals reaction and swelling of the layer. The swelling of the layer will cause it to crack and the fouling will be removed in lumps. The fast swelling of the fouling layer observed as soon as the alkali is added does not agree with the slow diffusion coefficient of hydroxyl ions ($D_{\text{eff}} = 10^{-8} \text{ m}^2/\text{s}$). The swelling process should be taking longer time if traveling through the fouling layer only. This indicates crack propagation (Figure 26) in the fouling surface, aiding the transfer of ions. The appearance of crack propagation suggests that the cohesive forces are stronger than the adhesive forces for the fouling made from pasteurization of milk.

With the alkali cleaning, most of the protein fouling is removed, but also some of the mineral fouling located closest to the surface. The major part of the mineral fouling is then removed in the acidic cleaning condition (Figure 27).
The model where the fouling cracks upon swelling and leaves the surface in larger lumps has been supported in a study by van Asselt et al in 2002 showing that approximately 30% of the inorganic deposit was removed during the first alkaline cleaning. They also showed that almost no organic compound could be detected by the turbidity measurement after the first alkaline and water rinse. The main inorganic part of the fouling was removed during the acid cleaning step [56].

One aspect discussed in the model by Jeurnink and Brinkman in 1994, is that the efficiency of the alkali cleaning agent concentration seem to have a maximum. A rubber like top layer was seen when cleaning the deposit at high concentration (4.5%) or at regular concentration but with reduced fluid velocity. This part of the experiment was conducted on the fouling produced in an evaporator at 70 °C and not in the heat exchanger. This has been noticed by other researchers and explained as due to a gel layer formed on top of the fouling layer that is not permeable for the disentangling or erupting polymer of the fouling layer (Figure 28) [16, 76]. Mercadé-Prieto et al in 2008 showed a retardation of cleaning when the gel was soaked in a high concentration (>3M) NaOH, but also that the effect is reversible if the alkali concentration is again lowered [76]. It should be noticed that these experiments are made in room temperature or slightly above (50 °C).
Figure 20 An increase of alkali concentration above the optimum causes a film to form on the surface of the fouling. This does not allow swelling of and diffusion through the protein deposit. This have a negative effect on the cleaning process [56, 76].

7.2.4 Grant et al 1999

There is no model to be found in literature that discusses the complexity of the mechanism involved in a type B fouling. The mineral deposit model produced by Grant et al in 1999 could be a complement to the type A fouling models seen.

A mineral deposit with radio-labeled calcium phosphate, P\textsuperscript{32}, was used as model fouling to detect and follow the dissolution of the deposit. The deposit was formed by drying a solution of calcium phosphate and calcium hydroxide inside the stainless steel tubes. The ratio of Ca/P was kept at the level found in fouling after high temperature processing of milk. The model discussed is based on the dissolving mass transfer flux, J, of a molecule due to the concentration gradient.

\[ J = -k_m(c_{\text{bulk}} - c_{\text{interface}}) \]

Where the \( k_m \) is the mass transfer coefficient and the rate of removal of the mineral deposit can be explained as

\[ r = -J(z)M_{\text{average}} \]

In the final model of mineral deposit removal, the process is a combination of shear forces, ripping the deposit in lumps from the surface, and dissolution, dissolving the deposit on a molecular level (Figure 29).
Understanding the cleaning process

Figure 21 Measurements of the removal rate of a calcium phosphate deposit onto a stainless steel. Both the mechanical shear from the flow velocity and the pH is of importance when studying removal of mineral deposit [91].

7.2.5 Xin et al 2004

Xin et al 2004 bases their mathematical model on a heat induced gel (and thus not necessary emulates protein layers formed under conditions close to those in a heat pasteurizer/sterilizer), the dissolution model for polymeric materials is integrated. Dissolution of polymeric material includes both disentanglement and diffusion through the polymer network, which was identified in the Gallot-Lavalleé and Lalande model from 1985.

Parameters that have an impact on the dissolution according to Xin et al are: the diffusion coefficient, D, of the cleaning solution, the molecular weight of the polymer, M, the quality of the solvent, structure and shape of the fouling layer, agitation from the bulk flow and the temperature [84].

The model introduced consists of two stages: swelling/uniform step and the decaying step (Figure 30). The reptation time of the polymer molecules, which is a delay time before the disentanglement of the polymer can be detected, is included in the model. The reptation and disentanglement is part of the swelling/uniform part of the model. The reptation event in the model has higher activation energy than the other processes, indicating that other mechanisms are involved.

During the swelling and uniform cleaning stage the rate of cleaning, \( r \), of deposit mass, M, is given by

\[
-r = \frac{r_m e^{k(t-t_r)}}{(\frac{\rho_m}{\rho_0} - 1) + e^{k(t-t_r)}}
\]
Here, $r_m$ is the cleaning rate during the uniform cleaning and is function of the mass transfer coefficient and volume fraction ($\Phi$) of disengaged polymers in the boundary layer. $\xi$ is the kinetic constant for the swelling process.

![Figure 22](image)

Figure 22 The cleaning process can be monitored by following the change in cleaning rate over time. During the reptation time, $t_r$, the cleaning rate is close to zero, then rapidly increases due to the cleaning agent/fouling reaction causing the protein layer to swell. $m_c$ in the decay stage correspond to the critical mass still on the surface [84].

After the uniform stage, the cleaning rate decay (decay stage) and at this stage the surface appears patchy and non-uniform. The cleaning is now dependent on the amount of fouling still left on the surface, $A_L$, but is still to be controlled by dissolution and mass transfer.

$$r = r_m \frac{A_L}{A_{L,0}} = r_m e^{-k_A(t-t_{su})}$$

$k_A$ is the first order rate constant that is dependent on temperature, flow velocity, cleaning agent concentration and the mechanical forces from the flow.

The three stages of swelling, uniform removal and decay was also reported by Gillham et al in 1999, who measured the CIP removal of WPC fouling (3.5 w/w %) produced at approximately 97 °C. The cleaning behavior was followed with the aid of heat transfer measurements as well as imaging where the surface structure and composition was followed over time [106].
Understanding the cleaning process

Table 9 Different models of mechanisms involved in the cleaning process. The deposits chosen range from those proceed from full milk to pure whey protein and pure mineral deposit.

<table>
<thead>
<tr>
<th>Author</th>
<th>Investigated deposit</th>
<th>Removal by shear forces</th>
<th>Bulk diffusion of cleaning agent</th>
<th>Internal diffusion of cleaning agent</th>
<th>Chemical and physical reaction leading to swelling</th>
<th>Cracks are forming allow more OH- to penetrate</th>
<th>Diffusion of deposit particles or dissolution (shredding)</th>
<th>The fouling layer breaks up and aggregates are removed. (patch wise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant et al., 1999 [91]</td>
<td>Ca/P</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Xin et al., 2004 [84]</td>
<td>Whey protein (gel)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
7.3 Conclusion for the understanding of the cleaning process

Several research groups have been trying to explain the mechanisms of cleaning in the dairy industry, mostly on type A, either by using fouling on pilot scale or a whey protein based model system. The important mechanisms for cleaning of fouling is

- Diffusion
- Swelling
- Disruption and dissolution
- Shear forces from the flowing liquid

Most of the protein models assume that the protein is removed patch wise after the fouling layer is swelled by treatment with an alkali detergent. The mineral content needs acidic conditions to dissolve. Elevated cleaning temperatures do not help remove mineral deposits due to the inverse solubility to several of the salts common in the milk fouling.

The formation of a gel on the fouling layer due to alkali treatment has been discussed and it has generally been found that 0.5% NaOH do not give an extensive gel layer, but do give sufficient cleaning. However, gel layer that retards the cleaning process if developed has not been observed in industrial processes. Based on previous studies, the gel seems to form

- At low concentrations, when the temperature is low (50 °C)
- At low concentrations, with reduced fluid flow
- At very high concentrations (NaOH at 4.5%)

For an industrial application where the temperature is between 85-120 °C, the concentrations at approximately 2% and normal production flow, gelation of the top layer of the fouling is not likely to occur.
8 Evaluation of the cleaning efficiency

Cleaning is a necessity in the industrial dairy production plant to sustain a safe product. The deposit forming on the surface in the heating sections decrease the heat exchange between the surface and the product. An efficient cleaning cycle is of importance in food production plants to prevent re-contamination of the product. In dairy production plants CIP (Cleaning-in-place) cycles are used, alternating NaOH or formulated alkali detergents and HNO₃ in a closed system. To measure the efficiency of the cleaning process is a challenge.

8.1 Industrial evaluation of the cleaning process

A clean surface in dairy processing industry is a surface with no microbial growth, but also no residues from the previous production run. Cleanliness can be evaluated in different ways; for example by quantify what is left on the surface after cleaning or by determine the amount contamination in the effluent.

Bacterial and fungal tests are often used for product samples to be able to locate contaminations and cross-contaminations; they are however not useful for the everyday cleaning evaluation, due to the time needed to for cultures to grow in the lab. ATP (adenosine tri-phosphate, an energy carrier inside the living cell) swabs and UV-light is faster and commonly used to locate problem areas and investigate newly installed equipment [109]. The interpretation of these methods is difficult and does not give an overall view of the state of the equipment. A visual inspection of tubes and heat exchangers can be used [110] and is thought of as being sufficient in many industrial applications, but more than a trained eye is sometimes needed to see the remaining fouling on the surface.

When using a system like CIP cleaning or as for the UHT plants a completely aseptic process, the equipment is not possible to dismantle for visual inspection after each run. It is therefore important for an industrial environment to be able to ensure that the cleaning process is potent enough to make certain that it is clean in every part of the system after each cleaning cycle.

The production process is optimized to have an as optimal schedule for production and cleaning as possible. Normally there are sensors for temperature, pressure, conductivity and flow available to assess the system. Fouling forming in the process equipment cause changes in heat transfer and the temperature difference between the wall and the product will increase. The fouling will also influence the flow behavior and pressure inside the tubes which can to some extent be monitored by the pressure and flow meter.
8.2 Evaluation of the cleaning process reveal the mechanism

For research purposes, the measurements do not need to be quick, easy to handle and non-invasive. The equipment can be dismantled and prepared at different stages and for different type of analyses.

Following the fouling removal (and buildup) with a direct online measurement with time on a surface inside a process plant is difficult to do on an industrial scale. Downsizing the equipment to a smaller pilot plant is many times used for research purposes, but still has not given the obvious methods to online and on site follow the changes of the fouling. The noninvasive effluent analysis are common in pilot scale cleaning studies [107]. By monitoring and analyzing the effluent during cleaning over time, important information is obtained; such as when the protein and mineral content is released in comparison to each other and to the current parameters used for the cleaning process. From effluent analysis, the original composition of the fouling can be determined, but without any spatial information. Since it’s common to foul the surfaces in a closed system, it is difficult to a priori visualize and determine the microstructure. The spatial information is therefore in many cases not available.

UV-absorbance can be used in cleaning studies to investigate the effluent concentration of proteins [84]. It is also possible to measure the protein content by measuring the chemical oxygen demand (COD) [5, 56] or the Kjeldahl method [31, 49-51]. An analysis of the effluent can only tell when there is nothing left leaving the equipment, but cannot validate that the surface is clean. Visual inspection is an off-line method and can range from ordinary photos to follow the course of cleaning, but also together with dyes for specific elements and microscopic techniques to enhance the details and resolution. Using Scanning electron microscopy (SEM) structural changes of the fouling, such as the swelling of the protein deposit, can be revealed. The content of the mineral deposit could be obtained in using a SEM-EDX, giving the elemental composition in a certain area of the sample [111].

The above mentioned methods are the same as discussed in chapter 4 under fouling characterization.

8.3 Methodologies for evaluating cleaning mechanisms and efficiency

The composition and structure of the fouling, before, during and after cleaning is important. But the questions on mechanisms and cleanability are important as well. The cleaning mechanisms presented in the models previously (7.2) could be verified by visualizing the processes and how parameters like diffusion, swelling, dissolution, cohesive and adhesive strength and mass transfer affecting them.

**Diffusion analysis by Fluorescence recovery after photo bleaching (FRAP)**

Diffusion in a material such as a gel can be followed by using a technique called Fluorescence recovery after photo bleaching (FRAP), which make use of fluorescent probes and the confocal laser scanning microscope. In this technique fluorescent probes in a defined area is
bleached out and the back diffusion of still fluorescing probes into this area is measured. The diffusion coefficient can be evaluated in a range of 0.1-300 μm²/s [112]. Thus it can be used to study hydroxide ions in an dilute alkali cleaning solution at 20 °C with an approximate diffusion coefficient, D = 1.65 μm²/s (7.1, p 37). With the effective diffusion of hydroxyl ions discussed by Jeurnink and Brinkman [56], the diffusion would be too slow to follow with the FRAP.

There is also the possibility of measuring the diffusion of a particle by the dynamic light scattering (DLS) technique. DLS works best when measuring dilute and transparent solution of spherical particles. Thus following the diffusion inside of a gel like structure, such as protein fouling, is challenging.

**Fluid dynamic gauging to follow thickness changes**

Fluid dynamic gauging is developed and used by the group of Professor Ian Wilson [108, 113-115]. With this technique, it is possible to measure the thickness of the fouling over time inside the system. The instrument is based on a suction flow through a nozzle lowered down toward the fouling surface (Figure 31). For the measurement to yield a good result the distance between the surface and the nozzle should not exceed one fourth of the nozzle tip diameter, the equipment therfore needs to be insertet close to the surface, with the possibility of obstructing a passing flow. The method has been used both for measurements under stagnant conditions and in fluid flow. Measurements with the FDG technique requires that the surface remains smooth and that it is not substantially removed in lumps by the force of the suction flow [108]. In addition to obtain the thickness of the fouling layer, analysis of the clean surface is need.

![Figure 23 Sketch of the principles of fluid dynamic gauging. The nozzle tip diameter is of importance since it will determine the sensitivity of the measurement. When the distance between the surface and nozzle (h) is within a distance of d/4 from each other, the accuracy of the measurement can be down to ±10 μm. Image adapted from [116]](image-url)
Ultrasound to follow thickness changes in-line and over time

Another method of measuring thickness and thickness changes is the use of ultrasonic waves. This method is possible to use in a way that will not disturb to the flow of cleaning agent solution. Ultrasonic methods use sound waves with a frequency above 20 MHz and two important parameters is the velocity and the attenuation of the sound waves [117].

Most ultrasonic devices uses piezoelectric crystal transducers that are both generating the ultrasound vibrations due to applied electrical potential, and also detect the (reflected) sound wave [117]. Thickness measurements of fouling in the food industry have previously been performed with the aid of ultrasonic sensor. Li and co-workers have studied the fouling of membranes [118, 119], whereas others like Withers and Lohr have studied the fouling in pipes [117-121]. In industry ultrasonic equipment is sometimes used for thickness measurement in pipes, monitoring corrosion and other similar phenomena.

There are mainly two types of ultrasonic transducers to be used for thickness measurements. The transducer can have the function of both transmitting and receiving the signal, a pulse-echo transducer (figure 32). There can also be use of two transducers, placed on the opposite side of the pipe from each other. In the latter case, one transducer is emitting the signal whereas the other is acting as a receiver [121].

![Figure 24](image) Schematic picture of how ultrasound can be used to analyze thickness non-invasively. The figure shows pulse-echo single transducer to the left. Here the energy waves from the echo of the opposite side of the pipe are used for analysis. To the right in the figure the transmission technique is shown, using two transducers for measuring the fouling thickness.

The main drawback of the pulsed echo system is that enough of the sound waves need to be reflected to make the analysis. An uneven surface of the fouling will spread the signal dramatically whereas the surface topography for the transmission technique doesn’t have a significant impact since the signal is going through the material. The scattering of sound waves, hence the lack of information reaching the receiver has been the main reason for not choosing the pulsed echo technique for thickness measurements [117, 120, 121].

It is not yet common to follow the thickness changes to quantify the cleaning. Another approach based on ultrasound to monitor the presence and removal of fouling from a surface is has been developed by the group of Professor Thomas Becker. The method makes use of acoustic impedance measuring the existence of the fouling in the production line from beneath the fouling suface. An artificial neural network has been adopted to analyze the data from the sensory system makes it possible to with high reliability determine whether the fouling is present or not [95]. The system is developed as a on/off system and is not possible to directly use to follow the fouling changes in discrete steps.
Evaluation of the cleaning process

By using the acoustic impedance, which is the reluctance to transmit the sound wave through the medium, the fouling can be visualized. The impedance, $Z$, is dependent on the density, $\rho$, and the velocity of sound through the medium.

$$Z = \rho c$$

The difference in density and velocity of sound between stainless steel, fouling and cleaning solution, should be possible to use to receive a good measurements of the changes within the fouling.

**Conductivity and turbidity**

Additional methods that can be used in the cleaning, to monitor the flow and concentration of cleaning agent, are as mentioned conductivity and the turbidity measurements of the solution.

Electrical conductivity (mS/m) will not give explicit information on the cleaning efficiency, but can give complementary information. Water, alkali and acid solutions will have different conductivity, and it is therefore possible to monitor the different steps of cleaning and correlate with additional measurements from another method. Conductivity is temperature dependent which has to be accounted for when comparing measurement data. Conductivity can be correlated to the concentration of cleaning agent and the pH at different temperature and can be useful during dosing of cleaning agent.

Turbidity is a sensitive method to use to detect particles in a suspension and has been used together with conductivity measurements to visualize when in the different cleaning steps the fouling is released from the surface. Salts that completely dissolve in the solution cannot be detected with this method. It is also more useful as an off-line method since the scattering from the released particles can be distorted by the foam forming in in-line cleaning solution [57].
Additional methods, mainly suitable for research are discussed in detail in chapter 4.3. Table 10 and 11 summarize methods can be used to follow and reveal the mechanisms of the cleaning process.

Table 10 Methods for evaluating cleaning efficiency and the mechanisms involved in cleaning, in-line measurements.

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive aspects</th>
<th>Negative aspects</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure changes</td>
<td>Hydraulic diameter</td>
<td>Non-invasive, common in industrial applications</td>
<td>Indirect measurement</td>
</tr>
<tr>
<td>Heat transfer changes</td>
<td>Temperature difference between surface and product</td>
<td>Follow overall heat transfer over time</td>
<td>Indirect measurement, Heat transfer coefficient for fouling is not constant</td>
</tr>
<tr>
<td>Fluid dynamic gauging</td>
<td>fouling thickness</td>
<td>Visualize the swelling and removal</td>
<td>Invasive equipment</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>fouling thickness</td>
<td>Good resolution of thickness changes</td>
<td>Heterogeneous surfaces scatter signal and reduce performance</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Changes in solution turbidity</td>
<td>Visualize small changes in particle concentration in the bulk</td>
<td>Sensitive to foam</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Follow changes detergent concentration</td>
<td>pH dependent</td>
<td>Temperature dependent</td>
</tr>
</tbody>
</table>

Table data calculated and provided by [source].
Table 11 Methods for evaluating cleaning efficiency and the mechanisms involved in cleaning, off-line measurements. The table focuses on research methods and does not include all industrial methods used.

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive aspects</th>
<th>Negative aspects</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-lamp</td>
<td>Product residues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Microbial growth</td>
<td>Quick test</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>structure</td>
<td>Structural information</td>
<td>Harsh preparations that could interfere with the structure stability</td>
</tr>
<tr>
<td>CLSM</td>
<td>structure</td>
<td>Structural and composition information</td>
<td>Require preparation of the sample</td>
</tr>
<tr>
<td>ESCA/XPS</td>
<td>Protein and mineral content</td>
<td>Information on both protein and mineral</td>
<td>Need dry samples. Some elements are difficult to distinguish</td>
</tr>
<tr>
<td>TOF-SIMS</td>
<td>Protein and mineral content</td>
<td>Information on both protein and mineral</td>
<td>Need dry samples. Some elements are difficult to distinguish</td>
</tr>
<tr>
<td>HPLC</td>
<td>Mineral content</td>
<td>Information on lactose and citrate content</td>
<td>No spatial information</td>
</tr>
<tr>
<td>COD</td>
<td>Organic content</td>
<td>Commonly used in research</td>
<td>No spatial information</td>
</tr>
<tr>
<td>UV absorbance</td>
<td>Protein concentration</td>
<td>Commonly used in research</td>
<td>No spatial information</td>
</tr>
<tr>
<td>Kjeldahl</td>
<td>Protein concentration</td>
<td>Accurate and well known method</td>
<td>Make use of toxic substance for catalysis</td>
</tr>
<tr>
<td>Atomic absorption</td>
<td>Mineral content</td>
<td>Many atoms can be identified</td>
<td>No spatial information</td>
</tr>
<tr>
<td>QCM-D</td>
<td>Fouling adsorption/desorption</td>
<td>Investigation on microscopic level</td>
<td>Only small amounts of mass</td>
</tr>
<tr>
<td>Ellipsometer</td>
<td>Fouling adsorption/desorption</td>
<td>Investigation on microscopic level</td>
<td>Only thin film on reflecting material</td>
</tr>
</tbody>
</table>
9 References


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