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Development of a dairy fouling model to assess the efficacy of cleaning procedures using alkaline and enzymatic products

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Abstract

Dairy fouling is defined as the accumulation of thermally insulating materials or deposits from process fluids which are especially formed on heat transfer surfaces. The selection of suitable cleaning strategies to remove dairy fouling requires the understanding of its composition and the relationships with the surfaces where it is formed. For the industry, the development of novel strategies to test cleaning products, as well reducing water and energy consumption during the dairy processing operations is of enormous interest. The results showed the development of a laboratory-milk fouling model (MFM) with an average content of 52.8 mg/cm² of fouling in the test coupons. Seven different cleaners were tested with a fouling removal effectiveness of between 55% and 97%. Additionally, for evaluating the cleaning process of the model, the turbidity of the cleaning solutions was assessed. We presented an enzymatic alternative to the use of traditional cleaning products, with a similar efficacy against the dairy fouling. 78% of fouling removal after the use of enzymatic solution, in comparison to the 72% of fouling removal after the use of alkaline cleaning products. A reduction in water (-33.3%) and temperature (-28.5%), as well as shorter cleaning times (-33%) than its chemical alternative, was observed.

Keywords:
Dairy fouling, cleaning, enzyme, Maillard reaction
1. Introduction

Fouling is generally defined as the unwanted accumulation of deposits on surfaces of interest. In the dairy industry, the problems caused by fouling are related to the inner surface of pipes, machinery, and the kind of treatment (De Jong, Waalewijn, & van der Linden, 1993; Barish & Goddard, 2013). In general terms, the problems caused by the presence of fouling can be classified into three different categories: operating problems, food safety, and product shelf-life (Bansal & Chen, 2006; Barish & Goddard, 2013). The operating problems related to fouling are blockages at industrial facilities or cross-contamination from batches of different food-products (Fryer & Asteriadou, 2009). These are particularly associated with heat treatments such as pasteurization where fouling could avoid the correct destruction of microorganisms in raw milk. One of the more serious issues of dairy fouling is that bacteria in milk have the ability to adhere to surfaces. This provides the conditions for the formation of biofilms in milk process tanks, milk process lines, and heat exchangers. Biofilms may contain spoilage and pathogenic microorganisms, resulting in a serious food safety issue (Bansal & Chen, 2006; Marchand et al., 2012; Gonzalez-Rivas, Ripolles-Avila, Fontecha-Umaña, Ríos-Castillo, & Rodríguez-Jerez, 2018). In those cases, microorganisms could either cause foodborne diseases or could reduce the shelf-life of the processed foods (Jindal, Anand, Metzger, & Amamcharla, 2018; Zouaghi et al., 2018).

In food processing industries this problem affects the day-to-day functioning (Takahashi, Nagai, Sakiyama, & Nakanishi, 1996). It has been suggested that the best procedure to clean the pipes after heating is a double cleaning process, using acid and alkali chemical products (Bylund, 1995; Graßhoff, 2002; Jeurnink & Brinkmann, 1994). However, it is not entirely clear which to apply first, the alkali or the acid chemicals. A two-stage cleaning process is sometimes inefficient and a clean surface may not be achieved (Timperley, Hasting, & de Goederen, 1994). Therefore, the cleaning of the facilities is an essential step to ensure an efficient process. Nevertheless, additional costs are required to eliminate cleaning chemicals and to neutralize chemically contaminated effluents.
(Changani, Belmar-Beiny, & Fryer, 1997; Graßhoff, 2002). Another approach for cleaning in the food industry involves the use of enzymatic products (Graßhoff, 2002; Turner, Serantoni, Boyce, & Walsh, 2005). This approach is often used to avoid polluting wastes and other problems that arise from the usage of corrosive products (D’Souza & Mawson, 2005; Potthoff, Serve, & Macharis, 1997). It has been found that certain cleaners damage both non-fouling coatings and food-grade stainless steel surfaces (Barish & Goddard, 2014; Jindal et al., 2018). Although, the use of enzymes could prevent these damages and prolong their utility (Potthoff et al., 1997).

The presence of carbohydrates is underestimated in terms of dairy fouling. It is controversial today as to whether the main component that starts the process of adherence to surfaces is the proteins or the calcium (De Jong, 1997; Jimenez et al., 2003; Visser & Jeurnink, 1997). However, during the maintenance or cleaning of the facilities from various fouling obstructions, it is seen that most of the material attached to the steel is brown in color (Barish & Goddard, 2013). This characteristic color is produced by Maillard reactions between the proteins and carbohydrates from milk, and could possibly be important elements of adhesion (Bylund, 1995). Consequently, this could be a good target to attack the problem. In fact, Takahashi et al. (1996) demonstrated that other compounds besides the proteins are attached during the heat treatment. There are two types of dairy fouling depending on the intensity of the heat in the process from which it is formed. For type A, the temperature range is between 75 °C and 110 °C and the composition is 50% - 70% proteins, 30% - 40% minerals, and 4% - 8% fat. Type B takes place at temperatures above 110 °C and the content is 70% - 80% minerals, 15% - 20% proteins, and 4% - 8% fat (Visser & Jeurnink, 1997). Furthermore, Bansal and Chen (2006) concluded that fouling of heat exchangers is a complex phenomenon and the mechanisms are not completely understood. It is believed that the formation of protein aggregates reduce fouling. However, the mass transfer of proteins between the fluid and heat transfer surface also plays an important role. According to this, different approaches have been suggested with the aim of creating a fouling model for the dairy industry to study its formation (Jun & Puri, 2005). In
this study, we focus on the fouling problems encountered in dairy industries. With our new method, we aim to design a protocol to produce fast and ready-to-use type A laboratory-scale milk fouling model (MFM), to test new enzymatic cleaning products, and find new ways of tracking the evolution of cleaning protocols.

2. Materials and methods

Two fouling formation models were developed, one for drying in open conditions and one for the recirculation of milk.

2.1. Source Materials

During this study, raw liquid bovine whole milk, refrigerated at 5 °C and supplied by a dairy farm (Granja Can Bordoi, Sant Antoni de Vilamajor, Spain) was used. Its composition was analyzed by Near Infrared Spectrometry (NIRS) using the model NIR 5000 (1100-2500 nm) (FOSS-NIR Systems Inc., Silver Springs, MD, USA). A total of ten samples were analyzed in triplicate (n = 30).

2.2. Open Drying Conditions Fouling Model

2.2.1. Container Surfaces

Stainless steel Type AISI 316 grade 2B is one of the main materials used for plate heat exchangers (PHE). Consequently, this material was employed as the reference for the study of fouling growth developing cleaning formulations (Barish & Goddard, 2013; Jimenez et al., 2013). In this case, square coupons of stainless steel that were 5 cm x 5 cm wide and 0.1 cm thick were used. The coupons were cleaned and disinfected according to the EN 13697:2015 standard (Anonymous, 2015). In order to retain a significant amount of fouling on a flat surface and prevent the loss of milk in each stage, auto-adhesive removable aluminum belts were used (Ceys, L’Hospitalet de Llobregat,
Spain), giving a box shape without a lid. Each one of the 4 pieces was 7 cm x 1 cm wide and 70 µm thick (Figure 1). Once the fouling formation process ended the aluminum belts were removed.

2.2.2. Fouling Formation
The containers (Figure 1) were weighed using an analytical balance (Mettler AE 100, Mettler-Toledo S.A.E., Hospitalet del Llobregat, Spain). To produce the MFM Type A (Figure 2A), the containers were pre-heated to 90 °C in a fan-assisted oven (IDL-FI-80, Labolan S.L., Esparzar de Galar, Spain). When the containers reached the desired temperature (90 °C), 3 mL of raw bovine whole milk was added to each container and then reinserted into the oven. Once the milk was air-dried on the surfaces, an extra 3 mL of raw milk was added, and dried again. This process was repeated to complete five cycles in total. Each drying cycle took 45 min.

The dried milk containers were then inserted into plastic flasks with 30 mL of deionized water at 50 °C. The containers were shaken using a vortex (REAX Top, Heidolph Instruments, Schwabach, Germany) at 2500 rpm for 1 min. The containers were then rinsed with deionized water in order to eliminate the unattached residues. Five more drying cycles and a final rinsing process was performed. Before determining the total fouling formed, the containers were dried to remove any excess water. The MFMs were weighed on an analytical balance before and after the aluminum belts were removed (Figure 1 and Figure 2).

2.3. Recirculation Milk Fouling Model
The methodology of Takahashi et al. (1996) was used with some incorporated modifications. Stainless steel discs of 2 cm in diameter were placed in the bottom of a Kitasato flask. Firstly, the stainless steel discs were cleaned and disinfected according to the EN 13697:2015 standard (Anonymous, 2015). In order to acquire room temperature, the raw milk was recirculated with the Kitasato flask using a peristaltic pump. The flask was immersed in a thermostatic water bath adjusted
to 90 °C for 18 h. The discs were then recovered and rinsed with deionized water. Finally, they were
dried and weighed using an analytical balance.

### 2.4. Cleaning Solutions (CS)

Seven cleaning products were used for the tests (Table 1). Two of them are already commercialized
products: a one-pass alkaline commercial product (CS1), currently used for removing fouling in the
industry and selected as the chemical cleaning product control, and one enzymatic product,
composed of protease, amylase and lipase (CS2). A non-foaming nonionic product was used as
surfactant (CS5). Taking into account the objective of this study, and the composition of the
commercial enzymatic product, we formulated four enzymatic solutions (CS3, CS4, CS6 and CS7).
The purpose of these was to act on proteins and carbohydrates in fouling, with enzymes developed
for the detergent industry: protease (Savinase®, Novozymes, Bagsværd, Denmark) and amylase
(Termamyl Ultra®, Novozymes). These enzymatic solutions were used with the nonionic surfactant
to increase the wettability and solubility of the residues in the aqueous medium. All enzymatic
cleaning solutions were concentrated tenfold compared to the working concentration, in 7.5 mL
sterile tubes and stored at -18 °C for the posterior use in the assays.

### 2.5. Milk Fouling Models (MFM) Cleaning Procedure

For each cleaning protocol, all the enzymatic cleaning solutions were thawed at room temperature
(18 °C - 22 °C). Then, they were diluted with 67.5 mL of deionized water adjusted to pH 9.5
(according to manufacturer's instructions to obtain the highest enzymatic efficiency), reaching a final
volume of 75 mL before being added to the MFM.

The cleaning solutions (Table 1) were placed in 160 mL plastic flasks containing the MFM (Figure
3A) and then sealed. All the plastic flasks were placed in a stirred thermostatic water bath (Unitronic
320 OR, J.P Selecta S.A, Abrera, Spain) at maximum stirring (111 units/min). For enzymatic
cleaning, the temperature was adjusted to 50 °C for 30 min (as indicated by the manufacturer in the commercial enzymatic product and followed for the other enzymatic formulas) in two 15 min phases.

For chemical cleaning methods, the temperature was adjusted to 70 °C for 45 min (as indicated by the manufacturer), in three 15-min phases.

For the enzymatic cleaning process (Figure 2B), the plastic flasks were placed in the stirred water bath for 15 min at 50 °C. The MFM was then removed from the cleaning solution and placed in a new plastic flask with 30 mL of deionized water at 50 °C and vortexed at maximum power for 1 min. This allowed the removal of the detached elements and simulated the liquid flow within the pipes in the facility. The coupon was then placed into the cleaning solution once again for 15 min. The procedure finished with another wash in water at 50 °C and an agitation for 1 min. The procedure for the chemical cleaning protocol was performed in the same way, but for 45 min in three 15 min phases in the stirred water bath at 70 °C. After each 15 min phase in the water bath, a washing step as in the enzymatic cleaning protocol was performed. After finishing the MFM cleaning procedures, the cleaned MFMs were then placed in an oven at 50 °C and weighed.

2.6. Monitoring the Cleaning Protocol

Tracking the cleaning processes of facilities is of great importance for possible future industrial application. Turbidity measurement appears to be an easy, low cost solution (Van Asselt, Van Houwelingen, & Te Giffel, 2002; Fickak, Al-Raisi, & Chen, 2011). For this purpose, a laboratory analysis using a turbidimeter in McFarland units (Densimat, bioMérieux, Marcy-l’Étoile, France) was performed.

2.7. Statistical Analysis

All the data collected from these protocols were processed using R free software (R Development Core Team). To compare differences between the variability of the average samples, one-way
ANOVA test was used with a posteriori contrast using the Tukey test. A $p$ value $\leq 0.05$ was considered significant.

3. Results and discussion

One of the main objectives of this study, when creating a new fouling model, was to reduce the technical requirements of other published methods and to focus on some variations that can easily be controlled. The advantages of simplifying the laboratory model can help with future research by speeding up the process of obtaining the model and requiring less resources for its production.

3.1. Drying Open Conditions Fouling Formation

The analysis of milk components shows a composition of $36.3 \pm 1.38$ g/L of fats, $33.8 \pm 1.01$ g/L of proteins, $56.31 \pm 1.89$ g/L of sugars and $126.4 \pm 1.9$ g/L of total solids, similar to a cow's whole milk standard as reported by Bylund (1995). The efficacy of the new proposed protocol of fouling production was calculated by the difference between the dry weight of the milk fouling attached at the beginning and at the end of the experiments. This procedure has been suggested in previous studies (Barish & Goddard, 2014; Liu, Jindal, Amamcharla, Anand, & Metzger, 2017). The results showed that the time to produce sufficient fouling to test new cleaning solutions was established in 8 h (10 cycles). Results revealed that after the ten dehydration cycles an average of $1.32 \pm 0.45$ g (52.8 mg/cm$^2$) ($n = 64$, surface of 25 cm$^2$) of fouling was obtained. The highest fouling layer previously reported was $19.21$ mg/cm$^2$ (Liu et al., 2017). Zouaghi et al. (2018) reported an accumulation of 30.8 mg/cm$^2$. However, they used a dilution of whey proteins and calcium as opposed to whole milk, therefore producing a fouling model over stainless steel of a grayish appearance. Additionally, the real fouling seen in the dairy industry has a caramelized aspect, with a brown color (Barish & Goddard, 2013).
In our study, a strongly attached, brownish-colored layer on the stainless steel surfaces of the MFM was observed (Figure 3A). That result was similar to previous observations obtained from real-life situations in dairy fouling (Barish & Goddard, 2013). The color may be related to a Maillard reaction between milk proteins and milk sugars, mostly lactose. The brownish color began to appear during the sixth cycle and small quantities of milk fat appeared as little droplets of clear liquid on the fouling during the drying process. According to our results, the Maillard reaction is a key element which may causes the adhesion of the fouling components. One of the most important stages of this protocol of MFM generation is the agitation step. The water rinses ensure the removal of proteins and other constituents of the milk poorly attach to the surface. Components that were retained in the surface received a higher thermal load, increasing the Maillard reaction, and leading to the formation of fouling.

3.2. Recirculation Milk Fouling Formation

Each stainless steel disc had a 6.9 cm$^2$ area on all sides. The total area of discs in this experiment was 55.26 cm$^2$ (eight discs), which was more than double the 25 cm$^2$ of the square coupons for the MFM. When using the alternative method to create a milk fouling model using a Kitasato flask, the milk showed a brownish after 18 h and all the inner surfaces of the system were covered in a thin layer of milk fouling. Once the discs were gently rinsed, dried, and weighed at room temperature, there was no appreciable change in their weight. When comparing the results obtained to produce the MFM using the drying method in open conditions and the method of milk recirculation, it can be seen that with less time and technical requirements a much larger amount of dairy fouling is generated on stainless steel.

3.3. Cleaning Efficiency
A cleaning agent that is currently used to clean milk fouling must be used as a reference when testing new formulas with a new model. In this case, two commercial cleaners (one chemically composed and one enzymatically composed) were used (Table 1). The results in Figure 4 showed that the effectiveness of the reference chemical cleaning agent CS1 for removing milk fouling was 73.31% and the outcome of the reference enzymatic cleaning agent CS2 was 77.99%. The MFM was tested with some new cleaning agents based on enzymes (Figure 3B), an environmentally friendly approach to the problem of fouling (Graßhoff, 2002; Boyce, Piterina, & Walsh, 2010). The advantages of using these products are mainly related to less wastewater production, reduced energy consumption by working at lower temperatures, reduced cleaning times, and less toxicity of the cleaning products by cleaning at a mild pH. They are also more environmentally safe because they are neutralized by biodegradation (Potthoff et al., 1997; Graßhoff, 2002; D’Souza & Mawson, 2005).

The enzymatic products leveled as CS3, CS6 and CS7 are shown in Table 1, composed by amylase, protease and surfactant, with a pH between 8.5 and 9.5 and tested at 50 °C, produced good results among the newly formulated enzymatic cleaners, with average effectiveness percentages of 75.35% to 80.43%. The formulas CS3 and CS7 had a similar minimum value, although CS7 had the best maximum value (Figure 4). Finally, the other new formulas, with efficiency percentages of 72.89% (CS4) and 69.5% (CS5) were tested at a pH of 9.5. After the cleaning treatment was performed (30 min), a large amount of the fouling formed on the coupon had been removed. A reduction near 70% of the fouling was ensured using any of the enzymatic cleaning treatments. This was achieved using lower concentration of enzymes an at lower temperature than is required in chemical protocols (Table 1). The products that contain amylase showed the highest values among the enzymatic ones, and the lowest pH values favored the elimination of fouling type A. After processing all the data, there were no statistical differences \((p > 0.05)\). This was a positive outcome for the fouling model in different conditions and cleaning solutions.
This demonstrates that using enzymatic cleaning products to attack this kind of residue in dairy facilities is a valid strategy. It can also be more economically beneficial than using chemical products due to the reduced energy costs of operating at a lower temperature (-28.57%) and the reduced number of rinse steps, hence producing less waste water (-33.3%), during cleaning protocols. Comparing the direct economic costs, the enzymatic products tested, represent an equal efficiency to the alkaline products, since a very low concentration of enzymes was used. The economic cost of the enzymatic treatment was calculated in 0.045 €/L. Alkaline chemical cleaning cost was estimated in 0.047 €/L. Consequently, enzymatic cost may be adjusted as a function of the enzymes selected, and its concentration. In the dairy sector, an average of 6.5 MWh and 2 m$^3$ of water is spent to produce one ton of processed milk. In this sense, a total of 98% of the water spent is of drinking quality and the 80% of the energy is for heating processes and cleaning operations (Vasquez, 2016). Other benefits of this system is reduced cleaning times (-33.33%), which is useful when aiming to shorten cleaning periods. Additionally, the system avoids the use of neutralization products before the cleaning waste is released into the sewerage system. Consequently, the correct use of enzymes offers a cost-saving alternative because they work effectively at low wash temperatures and mild pH. This allows reduced use of water, raw materials and energy, while improving the efficiency of cleaning and extending the useful life of the equipment. Additionally, it represents a considerable contribution to the recovery of the environment. Furthermore, recent trials with new chemicals or enzyme combinations promise an even broader application (Timmerman, Mogensen, & Graßhoff, 2016).

The pH range of the enzymatic activity was very effective in this cleaning protocol (Table 1) and was wide enough to see differences for future formulations. The products CS3, CS6, and CS7, evaluated at a pH 9.5, 9.2 and 8.5 respectively, and with the same formula, showed good average efficiencies. It is interesting to see that the laboratory-scale pH control is more accurate than the industrial scale indicating that these products could continue operating without very strict requirements. This information is useful because when digesting fouling proteins, functional groups
could be exposed and this may alter the pH of the medium, moving away from the ideal range for enzyme action. Additionally, the results with amylase and the color of the real fouling, alongside the laboratory one, help to support the theory about the presence of carbohydrates in dairy fouling. These data do not determine the role of caramelized carbohydrates, but simply knowing that it is present opens up new possibilities to attack and eliminate these residues that adversely affect the effective daily functioning of food companies. After this comparison, fixing a basic formulation for pilot plant scale trials should be possible.

3.4. Monitoring the Cleaning Protocol

Tracking the cleaning protocol with turbidity measurements was a quick and easy way to obtain immediate information about the process (Figure 5). At the beginning, the cleaning solution was translucent (0 McFarland units), but during the cleaning protocol it became turbid. During the agitation stages (Figure 2B), the water was full of detached pieces of fouling. Analyzing the turbidity is a simple index of the progress of the cleaning process, helping with optimization of this. Van Asselt et al. (2002) monitored the real-time turbidity by spectrophotometry of a cleaning solution to test the removal of protein fouling in an automated CIP system. Fickak et al. (2011) used the turbidity and conductivity measurements of the rinsing step to indicate the efficiency of the cleaning process completion.

4. Conclusions

A laboratory model of milk fouling has been developed. This artificial target (MFM) can be used for the evaluation of commercial and new cleaning products. This methodology has been demonstrated to be useful for assessing how effective the cleaning products are. New formulations using enzymes to attack dairy fouling have been proven to be a viable solution for this problem. No statistical differences between the cleaning solutions (chemical and enzymatic) were observed. Furthermore,
the use of new enzymatic solutions had the same effectiveness as chemical products, but with a reduction of water and industrial energy consumption. Turbidity measurement is an easy tool to track the cleaning processes used in the food industry, with minimum requirements of specialized workers and analytic techniques.

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References


<table>
<thead>
<tr>
<th>Cleaning solutions (CS)</th>
<th>Components and concentrations</th>
<th>Working temperature</th>
<th>Working pH</th>
<th>Cleaning time (min)</th>
</tr>
</thead>
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<tr>
<td>CS1</td>
<td>Higher recommended commercial alkaline cleaner dilution</td>
<td>70 °C</td>
<td>10 to 12</td>
<td>45</td>
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<tr>
<td>CS2</td>
<td>Higher recommended commercial enzymatic cleaner dilution</td>
<td>50 °C</td>
<td>9.5</td>
<td>30</td>
</tr>
<tr>
<td>CS3</td>
<td>1.2 mL/L protease 1 mL/L amylase Nonionic surfactant</td>
<td>50 °C</td>
<td>9.5</td>
<td>30</td>
</tr>
<tr>
<td>CS4</td>
<td>1.2 mL/L protease Nonionic surfactant</td>
<td>50 °C</td>
<td>9.5</td>
<td>30</td>
</tr>
<tr>
<td>CS5</td>
<td>Nonionic surfactant</td>
<td>50 °C</td>
<td>9.5</td>
<td>30</td>
</tr>
<tr>
<td>CS6</td>
<td>1.2 mL/L protease 1 mL/L amylase Nonionic surfactant</td>
<td>50 °C</td>
<td>9.2</td>
<td>30</td>
</tr>
<tr>
<td>CS7</td>
<td>1.2 mL/L protease 1 mL/L amylase Nonionic surfactant</td>
<td>50 °C</td>
<td>8.5</td>
<td>30</td>
</tr>
</tbody>
</table>

* Amount of nonionic surfactant for the products CS3 to CS7: 250 mL/L
Figure captions

Figure 1. Box-shaped container to form milk fouling made with stainless steel coupon and aluminum tape.

Figure 2. Schematic workflow. A) Milk Fouling Model (MFM) production on a laboratory-scale. B) Milk Fouling Model (MFM) cleaning protocol using enzymes.

Figure 3. Milk fouling Model (MFM). A) After the fouling formation protocol. B) After the enzymatic cleaning.

Figure 4. Efficiency of detaching milk fouling of different cleaning solutions (CS). CS1: commercial alkaline cleaner. CS2: commercial enzymatic cleaner. CS3 to CS7: new enzymatic formulas to test. In each boxplot, whiskers are the minimum and maximum value inside the 95% of the confidence interval for the median. Median is represented as a line inside of each boxplot. Efficiency is shown as percentage (0% to 100%). Each product was used in quintuplicate. No significant statistical difference were observed between products ($p > 0.05$).

Figure 5. Turbidity of different enzymatic Cleaning Solutions (CS) using the McFarland standard (each sample was tested in triplicate).
Highlights

- A laboratory model of milk fouling was developed
- Evaluation of commercial and new enzymatic cleaning products
- Enzymatic cleaners reduced the use of water and energy
- Turbidity measurement could be used to optimize the industrial cleaning procedures
**A**

1. Weigh
2. Add Milk
3. Drying
4. Agitate
5. Weigh

**B**

1. Weigh
2. Water Bath
3. Agitate
4. Weigh
1 **Highlights**

2 - A laboratory model of milk fouling is developed
3 - Evaluation of commercial and new enzymatic cleaning products
4 - Enzymatic cleaners reduce the use of water and energy
5 - Turbidity measurement could be used to optimize the industrial cleaning procedures

6

7

8