DIFFUSIVE MASS TRANSFER AND PROTEIN REMOVAL IN THE ALKALINE CLEANING OF A JELLYLIKE WHEY PROTEIN FOULING LAYER

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ABSTRACT

Single steps in cleaning a fouled heat exchanger surface eventually determine the cleaning kinetic and thus the overall performance of a cleaning process. Up to date the complex mechanisms of the mutual supportive processes in cleaning are not yet fully understood. This contribution presents a stepwise approach to investigate the individual process steps starting with the diffusion of the cleaning agent NaOH into a WPI gel, studied at stationary and flow conditions. In the experiments, temperature affected both the diffusion step and the subsequent removal but to different extents. This was further specified and it was found that there is an induction time for the release of disengaged proteins, accounting for the speed of the temperature dependent degrading reactions.

INTRODUCTION

Thermal treatment of milk is a necessary condition for safe food production. During this process milk fouling layers are being formed by pasteurization or ultra-high-temperature treatment (UHT), because the desired product temperatures exceed the denaturation temperature of whey proteins. Up to date fouling cannot completely be prevented thus requiring the cleaning of the equipment, in particular the heat exchanger surfaces. Cleaning processes are frequently oversized with a high consumption of energy and water which is disadvantageous for economic and ecological reasons. A sustainable process design can only be realized by an adaption of the cleaning process to the occurring soil [5]. Therefor the understanding of the underlying mechanisms limiting cleaning is a key aspect in achieving this. In the past decades numerous research projects were conducted to understand the individual steps in cleaning and whey protein-based deposits were widely used as a model food soil to mimic milk deposits. Besides the formation of fouling deposits from a whey protein solution on a heated surface also whey protein gels are being used intensively to study cleaning mechanisms due to their excellent and thus well controllable gelling behavior. According to the current state of knowledge, the underlying mechanisms in thermal denaturation of the protein molecules and the subsequent gel formation during thermal treatment, which occur during fouling and gel formation, are identical. Thus, rather homogenous gel soils are state of the art in cleaning research which can be easily shaped according to its application. [16]

The cleaning of whey protein-based gels is commonly conducted with sodium hydroxide (NaOH) and nitric acid is used when a noticeable amount of minerals (mainly calcium phosphate) has to be removed. During gelling, denatured and thus unfolded whey proteins (which is mainly β-Lactoglobulin, whereas α-Lactalbumin plays a minor role) are building covalent and non-covalent inter- and intramolecular bonds which form a three-dimensional solid network. Liquid filled pores are embedded in the continuous phase which consist of non-bonded proteins and solvent. This is also known as a hydrogel. In principle, all bonds that are formed during gelling, are to be broken during cleaning and the mass transfer processes enable the actual removal. Cleaning a whey protein gel can be simplified as a six-step process [17]:

1) The cleaning agent diffuses into the soil and causes
2) the gel to swell due to physical solvent uptake or inter and intra molecular bonds are broken due to chemical reactions, so degradation of the soil network takes place.
3) As a consequence, protein molecules disentangle and are disengaged for further removal
4) by diffusion to the soil-cleaning agent boundary.
5) The diffusion into the bulk fluid.
6) The removal by the fluid flow.

In the past decades intensive research was conducted to understand the interaction of the individual process steps to identify rate limiting steps which determine the overall cleaning kinetic [3, 4, 10, 12, 14, 17].

The diffusion of NaOH into the gel is the necessary condition for the subsequent dissolution and thus the cleaning of a foulant. The diffusion step is considered to be fast at low temperature but it was assumed, that it might be rate limiting when the

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cleaning rate is high, e.g. at high temperature [11] or high shearing conditions.
It was further assumed, that the chemical reactions, degrading the soil network are most likely to be rate limiting in overall cleaning [11] which could be approved and the breakdown of non-covalent interactions were identified to be rate limiting [4].

According to these findings, in the past a lot of work was done to understand the NaOH optimum in cleaning [3, 13]. In this study, the influence of the cleaning agent temperature is focused within the individual cleaning steps. Therefore, a manifold experimental approach is presented and a WPI gel is used to study the mass transfer into the gel under stationary condition, following [12], which was further applied on flow condition. The removal of disengaged soil fragments is studied using simple diffusion cell setups as presented by [2] and [7]. Since the cleaning steps described above are mutual supportive processes which cannot be considered nor investigated separately, the stepwise approach presented here points to the potential use of each method within the complex processes in cleaning.

EXPERIMEMNTAL PROCEDURE

Soil preparation

A whey protein isolate (WPI, Lacprodan® DI-9224, Arla) gel on stainless steel plates (20 x 80 mm, see Fig. 1) was used, as presented by [15] and applied according to [8]. A layer height of 3 mm was used for investigating the gel cross section. Gels were prepared by thermal denaturation of a WPI solution containing 0.15 $g_{WPI} \cdot g_{solution}^{-1}$. 1.4 $\mu L \cdot g_{WPI\ solution}^{-1}$ of a Thymolphtalein solution (1 % w/w in ethanol absolute) were added beforehand as pH indicator which enables visual side view observation of NaOH diffusion into the probes by a color change into blue at a pH above 9.3. The initial pH of 6.55 ± 0.055 was adjusted before usage to pH 6.7 with 1 M NaOH.

The thermal denaturation was performed in a closed aluminum mold in a drying cabinet. In a first preheating step, the mold was kept for 60 min at 60°C and subsequently the gelling was performed for 35 min at 80°C. Differential scanning calorimetry (DSC, DSC 3+ Stare System, Mettler Toledo) was used to measure the denaturation temperature of the WPI solution to 69.5°C as onset and 73.8°C as peak denaturation. A core temperature of the WPI solution of 69.5°C was reached after (84.4 ± 1.2) min, resulting in a denaturation time of (10.6 ± 1.2) min with a final temperature of (75.84 ± 1.33) °C. The temperature evolution in the gel was measured by a 1 mm type K thermocouple which was placed parallel to the sample plate. Its centered positioning was realized by the use of a small piece of hose (1 mm ID, 3 mm AD and a length of 10 mm).
The gelation was stopped by cooling the mold in a tap water bath. The gels were stored at 5°C until usage and were used within 5 d after preparation.

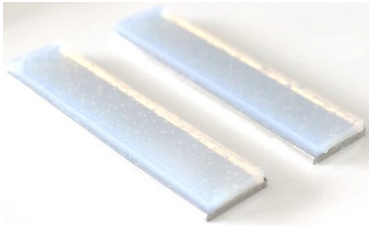

Fig. 1. WPI gel soil attached on a stainless-steel plate as model soil for cleaning investigation.

Diffusion of NaOH into the WPI gel

The diffusion of NaOH into the soil is investigated by a combination of the visual determination of the diffusive front with a simulation of the transport of hydroxide ions (OH) into the gel, as presented by [12].

The experimental setup for visual diffusion determination of NaOH into a WPI gel is shown in Fig. 2. The core item is the diffusion cell (1) which is a transparent rectangular cylinder made of PMMA where the gel sample (2) is placed on the bottom of the cell. The cell has a volume of 200 mL. Pictures for visual diffusion determination are made by an ultra-compact GigE camera (Mako G-503, Allied Vision, Germany) (3), equipped with a 12 mm objective lens (FL-CC1214A-2M, Ricoh, Japan). Lightning is realized by commercial LED tubes (4) in the front and one LED panel (5) from above. To exclude scattered light from the environment, the setup is placed in a closed box with a glass vision panel on the front. Separate boxes are used for detection (9) and experimental (10) section. A stainless-steel tank (6) is placed on the lid of the experiment section box for automated filling of the diffusion cell by a valve (7) at the start of the trial. Temperature dependent measurements are realized by preheating of the equipment (diffusion cell, tank and NaOH) to the desired temperature. Cooling is prevented by a fan heater (8).

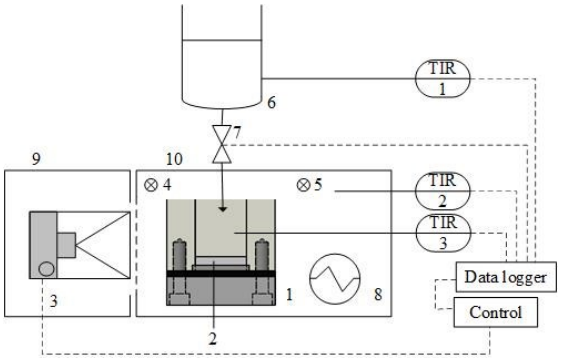

Fig. 2. Experimental setup for visual determination of NaOH diffusion into a WPI gel (numbered components described in the text).

The calculation of diffusion coefficients is a combination of Fick's first law with mass conservation described by [12] and further specified by [1], where time (t) and height (h) intervals are used to satisfy the following stability criterion:

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$$\frac{D_{eff} \cdot \Delta t}{h^2} = 0.5 \quad (1)$$

at a constant diffusion coefficient D_{eff}. The previous time interval is then used to calculate the hydroxide ion concentration $[OH]$ at a given depth x and time $t + \Delta t$

$$[OH]_{x,t+\Delta t} = 0.5\left([OH]_{x-h,t} + [OH]_{x+h,t}\right) \quad (2)$$

The calculation of the diffusion coefficients was performed with Matlab®.

Lab Scale Cleaning plant

Cleaning experiments were conducted using a lab scale cleaning plant, see Fig. 3. The core item of this plant is its transparent flow channel (1) with a length of 240 mm, made of polycarbonate, in which three soiled plates (each 80 x 20 x 2 mm) are placed in a row. The transparency enables optical access by a commercial digital camera (2) (Canon, EOS 77D) equipped with an objective lens (Canon EFS 18-55 mm/0.25m/0.8 ft). The cleaning fluid is circulated through the flow channel by a centrifugal pump (4) from a 5 L double jacket tempered glass tank (3). A constant volumetric flow between 1.3 L·min^{-1} and 1.6 L·min^{-1} is controlled by an inductive flow meter. The plant control is implemented in LabView (2015, National Instruments, USA). The cleaning progress can further be evaluated by post experimental protein quantification using a spectral photometer (Specord 210 plus, Analytik Jena) offline. A motor driven auto sampler (5) is installed for temporally high resolved sampling.

For visual investigation of the soil cross section a layer thickness of 3 mm was chosen. Compared to the height of the flow channel of 8 mm, cleaning leads to a large increase in the flow cross sectional area. Thus, two cases in the layer positioning can be distinguished. Either a planar overflow of the soil is realized in the beginning of a trial or in the end and the first case is investigated in this study. As a result, a dimple is formed during cleaning which is overflown by the core flow and cleaning is determined by diffusive mass transfer. Cleaning experiments are conducted at a constant flow rate resulting in a decreasing flow velocity and Reynolds number, respectively,

Cleaning experiments were conducted using 4 L of NaOH at 0.125 M NaOH at different temperatures (aiming at 25, 45 and 60°C).

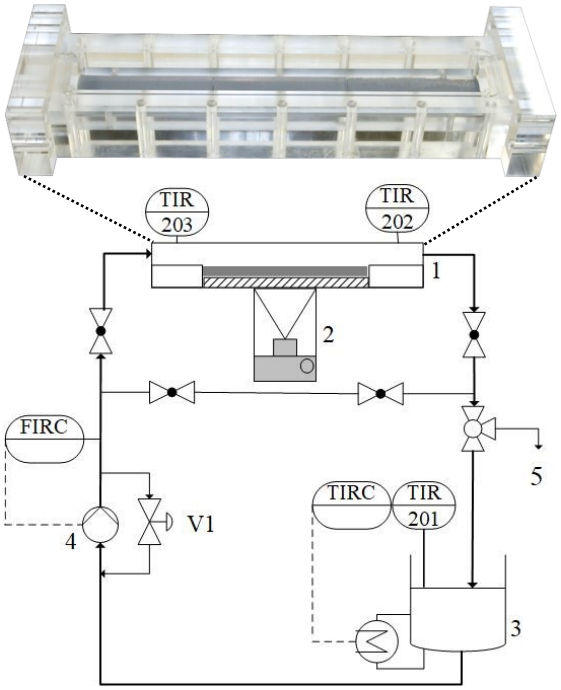

Fig. 3. Lab scale cleaning plant with the transparent flow channel for visual determination of layer height in side view.

Diffusion of disentangled WPI gel into bulk NaOH

To investigate the influence of diffusion of disentangled proteins from the top layer to the bulk solution, protein enrichment without external mixing was measured using a spectral photometer (Specord 210 plus, Analytik Jena), as described by [2]. A quartz absorption macro cuvette (Hellma, volume of 1750 µL, 50 mm path length) was used as diffusion cell and therefor the gel was cut in a rectangular piece of 9 x 49 mm and gently placed on the cell ground using a forceps. This is equivalent to a mass of (1.46 ± 0.09) g. For height dependent measurements, a motor driven cell shaft was built to enable the light pass through the cleaning agent at any position above the gel with a precision of 0.05 mm. A commercial cuvette holder is placed on a plate which can move up- and downwards. This is realized by a gear motor. Measurements and positioning are controlled by a LabView script (2015, National Instruments, USA).

The rectangular light beam with its original size of 8 x 2 mm (height x width) was focused using a 3D-printed diaphragm with a circular aperture of 1.5 mm. Absorption was measured at 283.5 nm and 15 mL of NaOH was used in each trial.

RESULTS AND DISCUSSION

Visual determination of NaOH diffusion

The supply of reactants within the soil is the necessary condition for cleaning and thus the diffusion of NaOH into the WPI gel is investigated

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in the following. A qualitative diffusion profile is illustrated in Fig. 4.

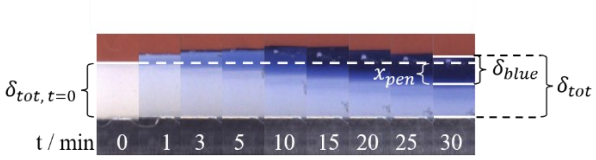

Fig. 4. Qualitative progress of stationary diffusion of NaOH into a WPI gel. Quantities derived from image processing are shown: total layer height δ_{tot}, blue penetration layer δ_{blue} and the penetration front x_{pen} calculated from this.

With the diffusion of OH ions into the gel, the initial pH of 6.7 changes therein. By use of the pH indicator thymolphthalein a blue penetration layer δ_{blue} is formed at pH 9.3 and larger. Over time, the penetration layer increases and also the overall gel height increases due to physical swelling, i.e. solvent uptake. After 15 min the release of degraded, loosen proteins prevails over the swelling and the layer height decreases.

The color intensity of the NaOH penetration layer increases in the course of an experiment. Chemically, the color intensity increases with the concentration of OH ions. In addition, also the experimental design contributes on it. Firstly, edge effects occur. The WPI gel layers are produced externally (cf. section 'Soil preparation'), cut out manually and the side edges therefore do not adhere to the wall of the diffusion cell. As a consequence, NaOH also gets between the gel and the wall. In side view of the pictures not only the desired one-dimensional diffusion direction (from top surface of the layer to its bottom) is realized but also from its front to its back. Secondly, the phase change from embedded proteins in the solid matrix to solvated and thus liquid proteins also influences the color intensity. In the stationary conditions of the diffusion experiment not only the first diffusion step of NaOH into the gels occurs but also the subsequent steps of removal (see section 'Introduction'). The disengaged proteins accumulate at the gel-solvent boundary and diffuse slowly into the NaOH bulk solution. As a consequence, a blue, protein rich but liquid layer arises which appears darker than the penetrated solid gel. The phase boundary cannot be distinguished by the color saturation separation presented in this study and the progress of NaOH penetration depth over time $x_{pen}(t)$ is thus determined by subtracting the change of the total layer height $d\delta_{tot}(t)$ at time t from the blue layer $\delta_{blue}(t)$ at time t.

$$x_{pen}(t) = \delta_{blue}(t) - d\delta_{tot}(t) \qquad (3)$$

The former comprises the total layer height at time t minus the initial total layer height $\delta_{tot}(t = 0)$.

$$d\delta_{tot}(t) = \delta_{tot}(t) - \delta_{tot}(t = 0) \qquad (4)$$

The actual gel layer thickness resulting from swelling and dissolution processes shall further be considered in future work.

In contrast, in the flow channel, the removal of the soil is already considered in the detected blue layer δ_{blue} and thus the difference from the initial total layer height $\delta_{tot}(t = 0)$ and the current total layer height $\delta_{tot}(t)$ is added to the blue layer to represent the penetration depth $x_{pen}(t)$

$$x_{pen}(t) = \delta_{blue}(t) + d\delta_{tot}(t) \qquad (5)$$

Pictures of the cleaning progress were taken with one frame per second and a resolution of 26 pixel per mm. Image processing was performed using Matlab®. The procedure in image processing is illustrated in Fig. 5.

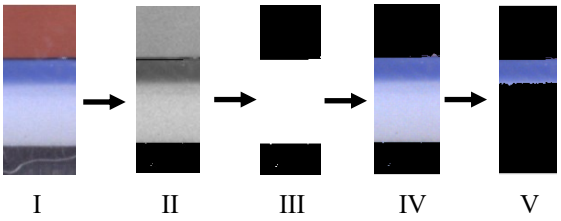

Fig. 5. Steps in image processing for penetration depth detection.

The original image (I) was transformed into a binary image for separation of the stainless-steel plate at the bottom of the image (II and III). After that, the background was separated from the gel layer using the Red Color channel in RGB color space (IV). To distinguish between the blue NaOH penetration layer (V) at the upper edge of the gel and the underlying unpenetrated layer (lower part in IV), a dynamic threshold setting was applied on each image using the saturation (S) channel in the HSV color space. As previously described in detail, during one experiment, the color intensity increases over time (see Fig. 4 and following explanations) and also the unpenetrated sublayer appears more bluish. These temporal changes require an individual threshold setting. The pattern of the pixel counts in the saturation channel also changes over time according to the changes in color intensity which is shown in Fig. 6. In the beginning of an experiment (at 0 min) the first peak is at S ≈ 0.05 and shifts slightly to the right with increasing trial time and broadens. A second peak at S ≈ 0.3 is small after 1 min and becomes larger as the color intensity increases. Two peaks thus separate the blue and unpenetrated sublayer and therefor the global minimum between the peaks applies as a criterion for a suitable threshold.

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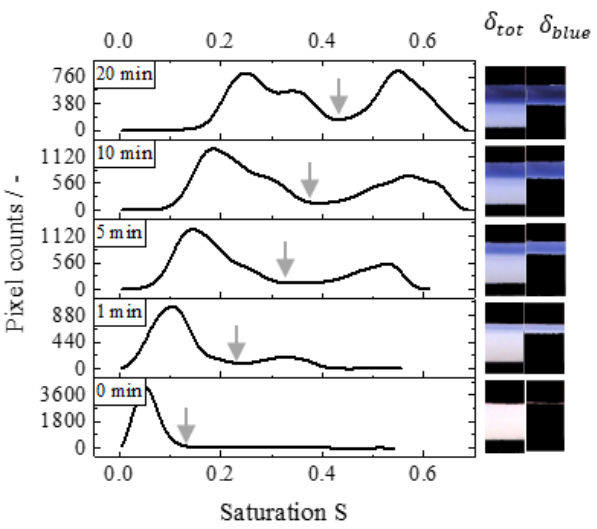

Fig. 6. Individual threshold setting in pixel counts of saturation channel for penetration depth separation from overall layer height. Arrows indicate the determined threshold value.

NaOH diffusion into the WPI gel

Previous studies found that the first diffusion step and thus the reactant supply within the soil is fast and therefore negligible at low temperature but might be limiting at high temperature [11]. Thus, diffusion is investigated at different temperatures in the following. Fig. 7 shows the experimentally determined continuously increase of the penetration depth x_{pen} over time of experiments at different temperatures ranging from (22.6±1.2)°C to (56.3±3.0)°C.

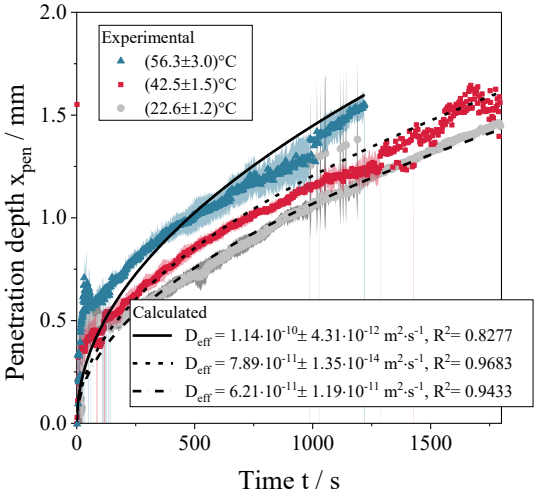

Fig. 7. NaOH penetration depth in a WPI gel at different temperatures. Three replicates (n=3) are shown for each temperature. Standard deviation is shown as error bars.

In addition, diffusion coefficients representing the experimental data were calculated to $6.21 \cdot 10^{-11}$, $7.89 \cdot 10^{-11}$ and $1.14 \cdot 10^{-10}$ $m^2 s^{-1}$ at 22.6, 42.5 and 56.3 °C respectively. As expected, diffusivity increases with temperature. The calculated profiles basically match the experimental data. At lower temperatures (22.6 and 42.5 °C) the calculated profiles represent the experimental data very well from ~200 s onwards. Before that, the above-described side effects and also the sensitivity in color detection leads to higher values than theoretically expected. The calculated diffusion coefficients represent the experimental data with coefficients of determination (R^2) of 0.9433 and 0.9683 respectively. At higher temperature of (56.3±3.0)°C a constant diffusion coefficient of $1.14 \cdot 10^{-10}$ $m^2 s^{-1}$ was determined with a significantly lower R^2 of 0.8277. The first ~470 s are underestimated and also the limits of detectability (as described before) contribute to the deviation. From ~470 s onwards, the calculated profiles overestimate the experimental data. In the calculation of the diffusion coefficients, a constant OH concentration at the gel-solvent boundary is assumed. In the experiments, the penetration depth x_{pen} refers to the initial height of the soil and its change (due to swelling and dissolution) is not considered here. During the course of an experiment, the gel dissolves from top to bottom and the disentangled proteins are in turn diffusively transported into the liquid bulk in the static test setup. Thus, in the experiments the OH concentration at the gel-solvent boundary is lower than theoretically calculated and the driving force for diffusion reduces in the course of an experiment.

Furthermore, the undefined temperature gradient between the tempered NaOH solution and the unheated soil has to be considered. The heat flow would probably not be sufficient to locally warm up the soil to the desired temperature of e.g. 60°C. Fig. 8 shows the experimentally determined continuously increase of the penetration depth x_{pen} over time of experiments with a target temperature of 60°C. Due to the experimental setup, the NaOH temperature within the diffusion cell can only be poorly controlled and the temperature decreases in the course of an experiment resulting in (56.3±3.0)°C and (59.6±2.5)°C for the studied cases of a non-preheated and preheated samples respectively. The sample preheating (equipment, environment and NaOH solution are preheating the same in both cases) influences the course of the penetration depth. A comparable diffusion coefficient of ($1.18 \cdot 10^{-10} \pm 2.35 \cdot 10^{-10}$) $m^2 \cdot s^{-1}$ was determined compared with the non-preheated sample of ($1.14 \cdot 10^{-10} \pm 4.31 \cdot 10^{-10}$) $m^2 \cdot s^{-1}$ but with a significantly higher R^2 of 0.9661. The experimental data of the first ~470 s is much better represented by the calculated data than in the case of non-preheating. A deviation arises of the calculated data from the experimental data at ~470 s onwards. The calculated profile in this case underestimates the experimental data. The preheating of the sample probably ensures that locally higher temperatures are reached (and kept) at the gel-solvent boundary compared with no preheating. It is known that dissolution and thus removal of the gel increases with temperature [11] which causes a local higher

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OH concentration as the gel-solvent boundary moves further. An accelerated driving force causes a faster moving of the penetration depth.

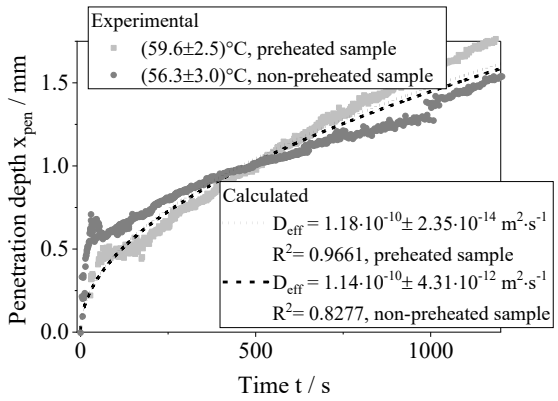

Fig. 8. NaOH penetration depth in a WPI gel with a target temperature of 60°C. Two comparing measurements with (n=1) and without preheating (n=3) of the sample and the calculated diffusion coefficients are shown.

The temperature dependency of the diffusion coefficient is shown in Fig. 9 and can be described by a quadratic function. From Fig. 9 it can be derived that the slight increase of the diffusion coefficient of the preheated sample compared with the non-preheated is mostly influenced by the deviating, higher temperature.

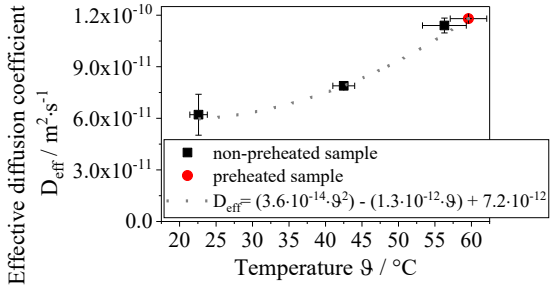

Fig. 9. Temperature dependent diffusion coefficients.

The determined diffusion coefficients (at ambient temperature, 22.6°C) are 16-30 times smaller than reported by Mercadé-Prieto et al. [11, 12] of 1 to 1.9·10^{-9} m^{2}s^{-1} investigating a whey protein concentrate (WPC) and a β-Lactoglobulin gel respectively. Besides the protein content (similar concentrations were used), also the gelation conditions (temperature and time) determine the structure and thus the permeability of the hydrogel. These are difficult to compare as different geometries and molds were used for gel preparation. In the context of gastric digestion, Luo et al. [9] investigated pepsin diffusion in WPI gels (also at 15 % (w/w)) and determined diffusion coefficients ranging from 1.9 to 3.9·10^{-11} m^{2}s^{-1} in very small samples (200 µL WPI solution in an eight well chambered coverglass, incubated for 30 min at 90°C). Taking this into account it is anticipated, that the method used to determine diffusion coefficients provides sufficiently accurate results.

Diffusion under flow condition

The first diffusion step was described in the previous section. In the following, the diffusion under flow conditions and thus the simultaneous removal will be considered to investigate whether the removal rate at high temperatures approaches the diffusion velocity.

Fig. 10 shows an exemplary trial at (23.5±1.7)°C and 1.3 L·min^{-1} corresponding to Reynolds numbers of 1000 to 1200. The reduction in the Reynolds number results from the enlargement of the cross-sectional flow area throughout the cleaning progress because a relatively high soil of 3 mm was used compared to the initial channel height of 8 mm in the beginning of a trial. Accordingly, also the flow velocity reduces from 0.096 to 0.07 m·s^{-1}. Two complementary methods for cleaning progress detection are shown in Fig. 10 a).

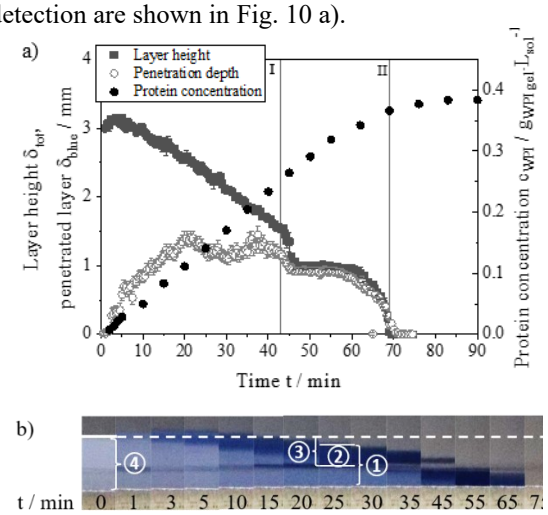

Fig. 10. a) Cleaning progress while cleaning a WPI gel with 0.125 M NaOH at 23.5°C and 1.3 L·min^{-1}. The visual measurement of overall layer height δ_{tot} (①) and blue NaOH penetration layer δ_{blue} (②) are shown. ③ is the NaOH penetration depth x_{pen} and ④ the initial layer height $\delta_{tot}(t = 0)$. Furthermore, the protein enrichment in the cleaning agent is shown. The bottom part b) illustrates the principal of side view photographs of the soil.

The protein enrichment in the cleaning agent follows a typical asymptotic profile and results in a cleaning duration of 83 min (where 99.9 % of the maximum concentration is detected). To investigate the influence of the first diffusion step of OH into the soil, visual side view tracing of the cleaning progress was performed and the resulting layer height δ_{tot} and penetrated layer height δ_{blue} are also shown in Fig. 10. In the first 5 minutes δ_{tot} increases from 3 to 3.15 mm due to a larger swelling than removal of the soil. The initial swelling is followed by an almost linear decrease up to 45 min (phase I

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in Fig. 10 a). The subsequent plateau illustrates the cleaning delay by the dimple formation outlined above. The layer height stays constant at 1 mm (one-third of the initial thickness) between 45 min and 60 min whereas the protein enrichment still increases linearly. Because deposit removal still occurs at a constant rate, the unchanging layer height is attributed to a larger swelling capability in this stage. Removal occurs by unlimited diffusive mass transfer of protein molecules into the fluid flow in this phase as the core flow does not reach the soil because of the dimple formation. The visual side view detection was identified to be reliable as described in the following. This is a rather one-dimensional detection method as aerial soil distribution becomes limiting at the end of the trial whereby protein release still occurs. It therefore results in a reduced cleaning time of 69 min compared to the protein enrichment in the cleaning agent. The diffusive front within the soil increases asymptotically until the soil is completely soaked with NaOH and δ_{tot} and δ_{blue} become identical at about 45 min.

Fig. 11 shows the evolution of the NaOH penetration depth while continuous cleaning in the flow channel. The cleaning curves aiming at 25°C, are flattening resulting from the mutual supportive processes of diffusion, swelling and removal. At higher temperatures (i.e. 45 and 60°C) no more flattening is observed as the dissolution is most probably more temperature dependent than the pure diffusion within the gel [11] and the penetration depth develops rather linear.

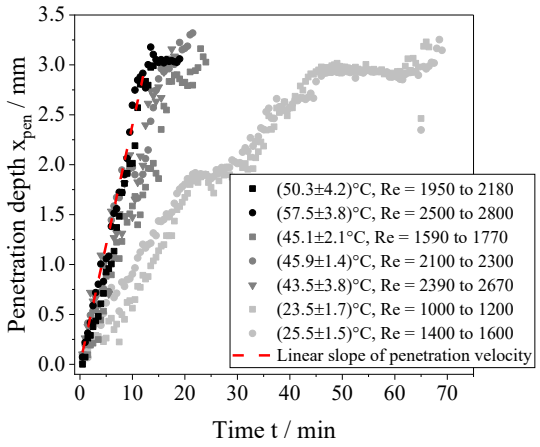

Fig. 11 NaOH penetration depth evolution during continuous cleaning in the flow channel.

Thus, the penetration depth velocity $\upsilon_{x_{pen}}$ is represented by the linear slope of the penetration depth evolution and is shown as dashed red line in Fig. 11. To distinguish between diffusion and removal, also the linear decrease of the overall layer height δ_{tot} is considered as constant removal velocity $\upsilon_{\delta_{tot}}$ (at high temperature, no initial swelling is measured; data not shown). The difference of the advancing velocity of diffusion $\upsilon_{x_{pen}}$ and the subsequent velocity of removal $\upsilon_{\delta_{tot}}$ is referred to as the limitation capacity of diffusion and its temperature dependency is shown in Fig. 12.

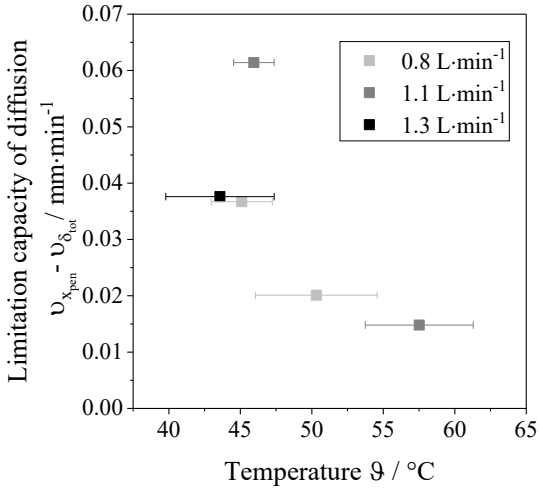

Fig. 12 Limitation capacity of diffusion is shown for constant diffusion velocities at temperatures larger than 40°C.

Data at low temperature (~25°C) are not considered as the penetration velocity is not constant. A clear temperature dependency derives from the presented data and it can be anticipated that the removal velocity does not exceeds and thus limits the diffusion velocity at higher temperatures than examined here. Both steps are mutual supportive. The driving force for diffusion is the concentration gradient of OH ions between the solvent and the gel. This reduces over time and a constant diffusion coefficient was measured in the stationary experiments when no (convective) removal occurs. In flow conditions, the concentration at the gel-solvent boundary is kept constant at a high level and thus the driving force for diffusion. As the diffusion step was shown to be fast and also temperature dependent, it is not assumed that the removal velocity would exceed the diffusion velocity only by increasing temperature. The flow velocity and thus the shear dependent removal has to be considered in future work as the mechanical impact in the removal step could only be investigated to a limited extent as the pumping capacity was limited. When comparing the penetration depth evolution at stationary and under flow conditions, it becomes clear that removal also highly depends on the applied shear stress. Fig. 13 shows the penetration depth at the moment when the entire sample is soaked with NaOH t_{soaked} during the flow experiments in comparison with the penetration depth which is developed in stationary conditions at the same time. The increase of the stationary penetration depth only depends on temperature. Due to the poor temperature control in the flow experiments it cannot be stated that a higher volumetric flow causes a higher penetration depth as it would be expected. The highest liquid speed ranging from 0.09 to 0.014 m·s^{-1} results in the

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smallest penetration progress but was also conducted at the lowest temperature. Further experiments are required to distinguish between the influence of temperature and shear on the soil removal.

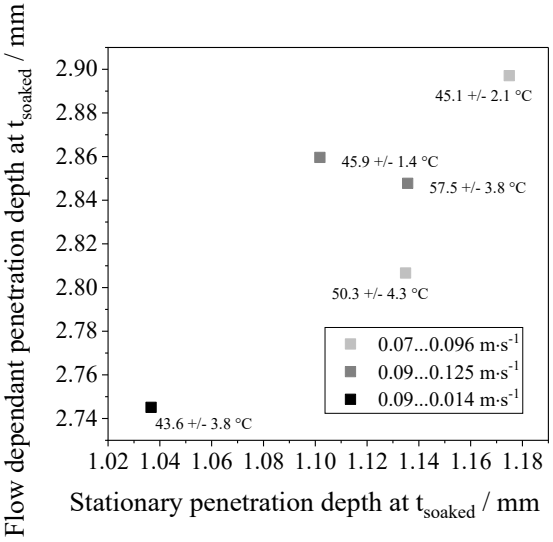

Fig. 13 Constant NaOH penetration layer dependency of temperature and volumetric flow.

With the clear detection of the current layer height during continuous cleaning, a constant penetration layer develops as presented by Mercadé-Prieto et al. [12] and is shown in Fig. *14*. This refers to a steady state of cleaning where both, diffusion and removal velocities are constant and the measured values in this study correspond very well with the values presented by [12], ranging from ~0.65 to 1.05 at 22°C and ~0.35 to 0.55 at 60°C. The range refers to different materials studied. During continuous cleaning the phase of constant NaOH penetration layer ends when the remaining soil is completely soaked with NaOH. The first diffusion step is completed then and subsequently the cleaning only depends on the removal of disentangled proteins.

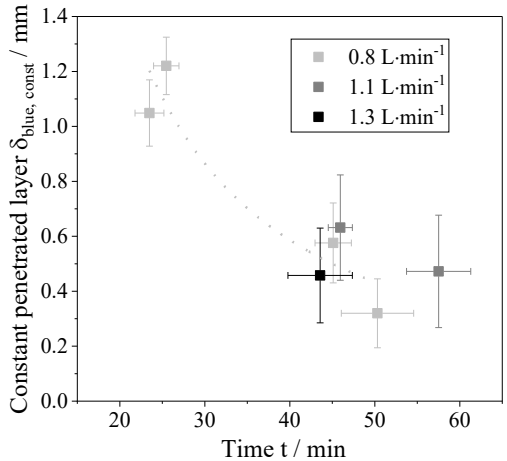

Fig. 14 Constant NaOH penetration layer dependency of temperature and volumetric flow.

Furthermore, experiments at higher temperatures are required to verify that the removal velocity does not approach the diffusion velocity at higher, more industrial relevant cleaning temperatures. Also, the influence of the shear dependent removal is of interest.

WPI release from soil and diffusion within NaOH

Within the investigated parameter range, so far it was not shown that the first diffusion step of NaOH into the WPI gel becomes rate limiting when removal is approaching the diffusion velocity, i.e. at high temperatures or high shear forces. In the following, the removal step and subsequent transport within the cleaning agent is investigated. Fig. 15 shows concentration over time profiles of the protein enrichment under stationary diffusion conditions. The different distances above the soil result in similar profiles which indicates, that the diffusion of detached proteins within NaOH is fast and a maximum distance of 15 mm was not high enough to measure transport velocities from different height measurements.

The first release is measured after 4.5 min at all distances with 0.055 $g_{WPI}{\cdot}L_{sol}^{-1}$ which is 1.5 % of the maximum reached equilibrium concentration. In comparison, 7.4 % of the soil were removed by the flow in the run shown in Fig. 10 at the same time. The measured profiles reach a constant end value which is about 3 % of the maximum possible concentration that would result after infinite time when the entire gel is totally in solution and complete mixing has taken place. The linear measurement range for direct protein quantification accounts to 6 $g_{WPI}{\cdot}L_{sol}^{-1}$ and therefore an extension of the experiment significantly longer than 24 h could be possible to quantify the measured equilibrium between upcoming detached protein molecules from the soil and releasing molecules to the upper, protein free part of the liquid phase. With the existing setup a local decrease due to complete mixing after infinite time is not expected because of the high amount of gel inserted in the small cuvette of (104.48 ± 6.64) $g_{WPI}{\cdot}L_{sol}^{-1}$. Rather stepwise plateaus are expected due to continuous release of proteins. A quantification of the diffusion process by determining a diffusion coefficient is not possible in this setup because unlimited diffusion to the upper end, the so-called infinite half space, is not fulfilled. However, it is very well suited for the determination of the initial soil removal which is assumed to be material specific and according to the findings in this study also temperature dependent. Further experiments are required for more detailed insights.

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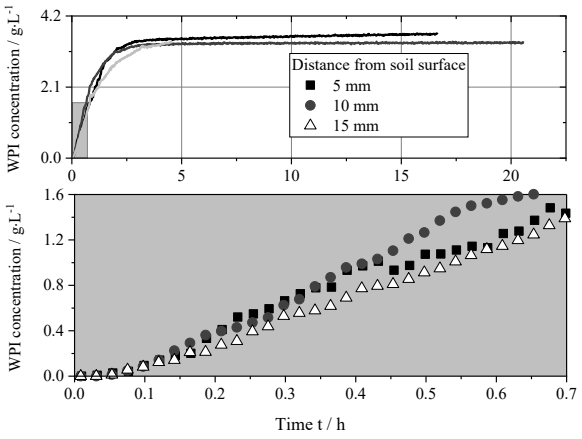

Fig. 15. WPI release from the soil network and diffusion within NaOH measured by spectral photometry and a cuvette as diffusion cell.

To quantify the transport within the NaOH, a further setup for diffusion measurements was used in accordance with [7]. The diffusion cell used for the visual measurement of the first diffusion step was identically constructed and built from stainless steel. It was equipped with a lid that holds three syringes with stainless-steel cannula tips in different lengths for simultaneous sampling and subsequent protein quantification, as presented by [6]. In this setup a maximum concentration of 22.5 $g_{WPI} \cdot L_{sol}^{-1}$ could be reached after infinite time and complete mixing, using 200 mL of NaOH. The protein enrichment of WPI after 15 h of release and diffusion in NaOH is shown in Fig. 16 a). Different concentrations of NaOH as well as water were used as cleaning agents. The highest concentration was measured at the closest distance to the soil surface which was 6 mm. With increasing distance this is further reduced by diffusion. The largest distance was 20 mm at which about one third of the highest concentration was measured. This is either due to the shortage of distance and a high release from the soil or accounts to the suction effect of the syringe whereby a volume of about 100 µL was taken up. A desired one-dimensional concentration measurement in a point is not met in this setup.
Even though water alone is not able to disturb the soil network but leads to swelling, comparable concentration profiles were measured in all cases. The fluid filled pores of the soil structural network are enlarged by physical swelling and protein release and thus diffusion occurs from the top layer by damage of the network walls. The widely discussed optimum in NaOH concentration for cleaning whey protein-based soils which is reported to be 0.1-0.2 M (this corresponds to 0.4 to 0.8 % w/w) at low temperature up to 70°C [3] was found here as well. 0.125 M NaOH results in the highest concentration near the soils surface (at the smallest distance of 8 mm) which can be assumed to rather account for a fast release than for a fast transport by diffusion. The concentration profiles resemble each other already from a distance of 10 mm and the subsequent diffusional transport within the following 10 mm is similar. Previous findings, that the chemical impact in terms of OH ion concentration is key limiting by breaking down interprotein non-covalent interactions [4] can be found here in terms of the concentration dependency of the initial released amount of proteins at 8 mm.

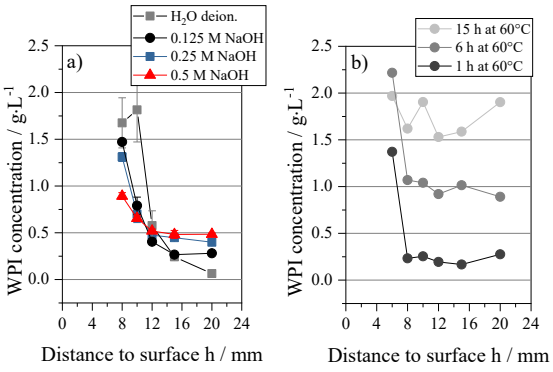

Fig. 16. WPI enrichment after 15 h of diffusion time at different distances to the soil surface. A diffusion cell with sampling and external protein quantification was used.

To evaluate the temperature influence in the release and removal processes, measurements with the syringe setup were also conducted at 60°C as shown in Fig. 16 b). After 15 h of diffusion, the surface close concentration increased from 1.5 $g_{WPI} \cdot L_{sol}^{-1}$ at 25°C to 2 $g_{WPI} \cdot L_{sol}^{-1}$ at 60°C. And in turn the concentration profile at 25°C after 15 h is comparable to that at 60°C after only 1 h. After a significantly higher concentration in protein supply, the concentration measured at further distances is almost constant within one time set and also applies for shorter diffusion times of 1 h and 6 h. The abrupt drop in concentration can clearly be seen after 1 h and 6 h whereby the concentration is rather constant after 15 h. Thus, temperature contributes to the steps of chemical reaction in breaking down inter protein cross links rather than accelerates the disentanglement of proteins as it is assumed that the disengagement would lead to fast diffusional removal.

CONCLUSION

An investigation of the individual steps in cleaning a whey protein isolate gel with NaOH is presented in this study. The diffusion of NaOH into the gel was determined visually resulting in diffusion coefficients ranging from $0.6 \cdot 10^{-10}$ to $1.1 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ at ~22 to 60°C respectively. The temperature dependency of the first diffusion step could be shown as expected.

For further investigation of the temperature influence of the first diffusion step, cleaning experiments were conducted in a lab scale cleaning plant and a diffusion front was clearly developed at a low temperature of 25°C and was faster removed

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at higher temperature. The contribution of accelerated removal due to temperature and shear stress could not be individually quantified and it is not anticipated that the removal velocities would approach the diffusion velocity.

The removal was further investigated under stationary conditions using a macro cuvette as diffusion cell and direct absorbance measurement in a spectral photometer. An induction time of first protein detection of 4.5 min was measured accounting for a release velocity of disengaged proteins. The differentiation between release and further movement within the cleaning agent was realized using a larger diffusion cell. Clear differences in the amount of released protein were detected at varying NaOH concentration, whereby the molecular movement within the cleaning agent was comparable for all concentrations. Temperature led to an increase in protein supply as well as the molecular movement and is therefore concluded to play a central role in acceleration of protein disentangling as well as the movement of disengaged proteins.

Further experiments will be conducted at higher, more cleaning relevant temperatures. Furthermore, the network degradation has not been investigated so far and is going to be subject for further investigations. With it, a methodological approach can be presented to track down the individual steps involved in cleaning that can be applied to identify the relevant rate limiting step under different cleaning condition.

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NOMENCLATURE

Abbreviations

DSC	Differential Scanning Calorimetry
HSV	Hue Saturation Value color space
NaOH	Sodium Hydroxide
OH	Hydroxide ion
RGB	Red, Green, Blue color space
WPI	Whey Protein Isolate
SMUF	Simulated Milk Ultra Filtrate
UHT	Ultra High Temperature Treatment

Symbols

c	Concentration, mol L^{-1}
D	Diffusion coefficient, m^2s^{-1}
h	Height (interval), m
Q	Swelling ratio, no dimension
S	Saturation channel in HSV color space
t	Time (interval), s
y	Distance, m
x	Depth, m

Greek symbols

ξ	Dimensionless concentration, no dimension
δ	Layer height, m
Δ	Difference, dimensionless
ϑ	Temperature, °C

Subscript

A	component A
B	component B
eff	effective
pen	penetration
sol	solution
0	initial condition

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