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Research Article

A Microbiological Test Method to Determine the Cleanability of Filter Media in Solid-Liquid-Separation Applications

One of the major properties in the design and development of filter media for the food and drug industry is their cleanability. They should be designed according to hygienic criteria thereby decreasing residuals and reducing cleaning time and costs. A reproducible test method is necessary to determine or validate the degree of cleanability of filter media and to indicate areas of poor hygienic design. This work focused on the development of such a filter test and provides information about impurity, whether it occurs or not, and its local resolution. The growth of a microorganism, which is introduced as impurity, is used for the detection of residues which grow to the surface of the sample and offer a 2-dimensional analysis. With this approach, different kinds of filter media can be compared with each other regarding their cleanability.

Keywords: Cleaning methods, Microorganisms, Separation techniques

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1 Introduction

Special requirements exist in the food and pharmaceutical industry concerning the cleanability of the equipment used [1–4]. The manufacturer and later on the producer have to take care of several hygienic and sanitary standards or directives which are published and controlled by authorities (e.g., the European Community or the Food and Drugs Administration) [5]. Recently the focus has changed regarding the development of new components from applications with aggressive cleaning procedures to milder cleaning methods. This is achieved by using more sensitive products and increasing the requirements in consumer protection [6]. The hygienic design criteria increases the cleanability of components and process facilities, which means microbiological impurities or other contaminations are avoidable and do not lead to high costs [7].

The inactivation of bacteria is necessary and is accomplished by using a special cleaning detergent or sterilization step with high temperature. Additionally the prevention of adhesion of bacterial residues is important. After an insufficient cleaning procedure, the bacterial fragments will accumulate within the components and build up a kind of biofilm, which creates a nutrient medium for the bacteria [5]. In this case, the growth of other organisms will be promoted. This is the reason why it is absolutely necessary to design individual components or machine parts, using the hygienic design criteria, which make adhesion more difficult [8].

The filter medium is one of the weak spots during the cleaning procedure of machinery. Cleaning such parts is very challenging, especially in a cleaning-in-place step (CIP), because most of the structural design is due to the material properties or its design is unsuitable for the entire removal of residues. Until today, neither standards nor criteria exist, how a filter medium has to be built to achieve simple and complete cleaning. Therefore, in critical manufacturing plants one-way or singly used filters are state of the art.

Due to financial reasons, the filter medium has to be cleaned and reused for special applications. Additionally, replacing the filter medium during the production process, especially in a centrifuge, leads to a loss of production time. In such a multipurpose application not only one product is produced. Hence, an entire cleaning procedure is essential to avoid cross contamination and to decrease the loss of production time.

A reproducible filter test is inevitable to improve the cleanability and to develop new kinds of filter media [9]. This test would provide a possibility to obtain information about the degree of cleanability and enables to identify the best filter medium for cleaning. The microbiological method, which is developed in this work, is based on the EHEDG (the European Hygienic Engineering and Design Group) test method [10], but with significant modifications regarding the used parame-

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ters and apparatus and the assessment of cleaning. The main steps of the new test method are:
– Well-defined contamination in a stainless steel apparatus with *Bacillus stearothermophilus* var. *calidolactis* as the test strain
– Cleaning in a nozzle cleaning system with purified water without any detergent solution
– Preparation with Shapton & Hindes Agar
– Incubation in an incubator at 60 °C for 18 hours with digital pictures taken during growth
– Assessment of the degree of cleanability with a computational analysis of digital pictures

The test is applicable for different kinds of stainless steel multilayer filters, a woven wire cloth medium. The difference between the five filter media tested is the design of the support layers on the backside of the medium. At first, the test should provide information about the influence of the supporting layers on the cleanability of these different filter media.

2 Materials and Methods

Tested Filter Media and their Preparation

The deployed filter medium is a woven wire cloth medium with an effective pore size of 10 µm in the filtrating layer and a very small filter resistance. This medium consists of seven sequentially piled sintered layers. The second layer is the filtrating layer which is protected by the guarding layer with a wide pore size (see Tabs. 1 and 2). The subsequent layers on the backside of the filter layer in flow direction are supporting layers with increasing mesh size and different design for testing (see Fig. 1). The mechanical stability of this multilayer metal filter needs to be high, because of the mechanical stress of the filter drum during application. This is one of the main advantages of the filter medium. In that case, the woven wire cloth has to be reinforced with welded struts on the backside of the self filtrating filter drum.

The main advantage for the cleaning of this filter medium is the flat set-up, which takes little volume within the apparatus. Small quantities lead to a small intrinsic surface. The smaller the intrinsic surface of a filter medium, the lower the probability for adhesion of particles or residues after a cleaning step. That is why a surface filtration is generally favored, compared to a depth filtration.

In order to simplify the handling for testing of the unique media, the medium gets a round circular shape with 52 mm diameter and an area of 2124 mm^2 (see Fig. 2). To carry out this process without risk, the above mentioned limitations are vital.

To characterize the five tested filter media, a special parameter, the wet-bulb volume (V_b) is introduced. It represents the volume of the liquid, which is absorbed after a well-defined moisturization method regarding a single sample. A completely dry filter sample is weighed and is placed in a glass with purified water. Afterwards the glass bottle is set inside an autoclave

Table 1. Characteristics of the different filter media P1–P5 (layer type Haver & Boecker/Germany) – seven layers.

	P1	P2	P3	P4	P5
1st	square	square	square	square	square
2st	DTW 18	DTW 18	DTW 18	DTW 18	DTW 18
3st	square	square	square	square	square
4st	SPW 300	SPW 300	SPW 300	SPW 300	square
5st	square	SPW 300	SPW 300	SPW 300	square
6st	DTW 18	SPW 300	SPW 300	SPW 300	square
7st	square	square	square	square	square
				fifth layer 90° turn	

Table 2. Mesh size of each layer in µm (Haver & Boecker/Germany) of the filter media P1–P5 according to DIN ISO 3310-1.

	P1	P2	P3	P4	P5
1st	400	400	400	400	400
2st	18	18	18	18	18
3st	400	400	400	400	400
4st	300	300	300	300	630
5st	400	300	300	300	1250
6st	18	300	300	300	2000
7st	3150	1250	1250	1250	3150
				fifth layer 90° turn	

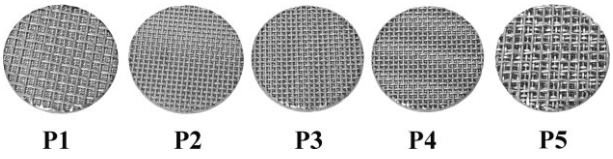

Figure 1. Back view of the five tested filter media with a different design of the supporting layer.

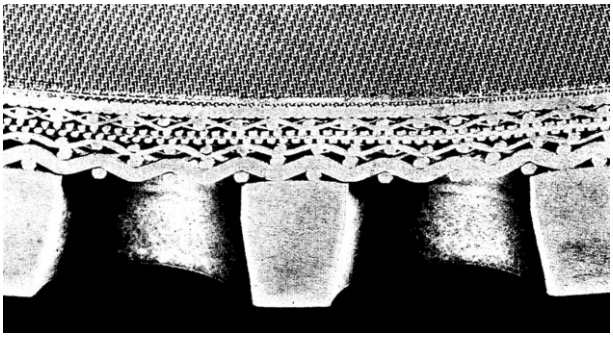

Figure 2. Cross sectional view through the filter medium with a perforated plate bottom sided.

which is evacuated at 15 mbar to supersede the air volume in the interstices. The sample remains for 30 min under these conditions and is than taken out and weighed again. The wet-bulb volume is defined as follows:

$$V_b = V_d - V_m \quad (1)$$

This value V_b is obtained by measuring the weight of the sample before (V_m) and after (V_d) moisturization.

The results of the microbiological cleaning test indicate a relationship (or causality) between the cleanability and the wet-bulb volume.

Experimental Setup

A major feature of the proposed microbiological test method is the detection of residues by discoloration that is caused by the microorganisms in a nutrient medium. The aerobic, thermophilic test organism *Bacillus stearothermophilus* var. *calidolactis* has been chosen for this method, because of its high growth temperature (60 °C). This offers the opportunity to carry out the test under non-sterile conditions. Very few organisms can grow in such an environment. Also, an impact caused by other kinds of microorganisms is negligible. Furthermore, it is a fast growing microorganism with a generation time of 11 min. First visual results can be noticed after approx. six hours.

The entire microbiological test method is divided into five consecutive steps and starts with a well-defined contamination. Fig. 3 shows the schematic flow chart of the contamination step.

Prior to testing, the equipment is sterilized. Afterwards the completely dry filter sample is mounted into the contamination device and the spore suspension is passed through. The suspension consists of purified water with a concentration of 15,000 *Bacillus* spores/mL. The contamination is carried out with a volume flow of 0.8 L/min and a temperature of 22 °C. The hose pump is able to pump the suspension forwards (15 min) and backwards (15 min) within the loop. The suspension is then drained and the complete test section is flushed with 1.5 L purified water and a volume flow of 1.0 L/min.

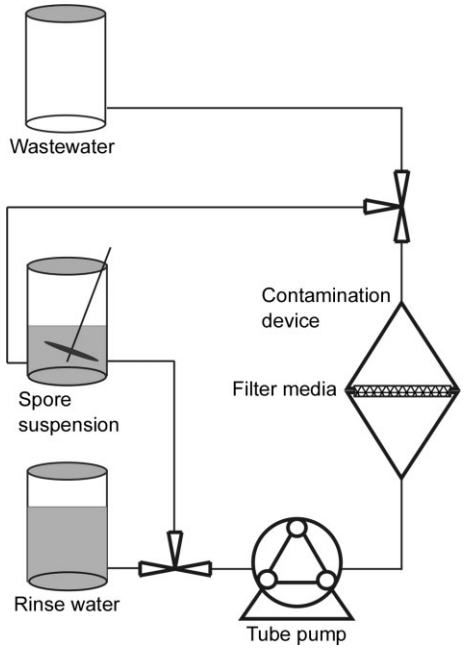

Figure 3. Schematic representation of the contamination device.

After contamination, the cleaning procedure starts with installing the filter sample within the nozzle cleaning system. The medium is placed in a special mounting located between two glass segments (see Fig. 4). The mounting can be turned and horizontally moved through different nozzle flows. It is possible to use different nozzles and therefore influence the result of cleanability. A flat jet nozzle showed the best cleaning results.

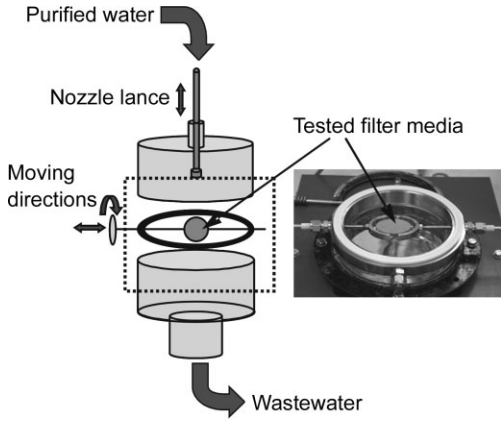

Figure 4. Nozzle cleaning system with a mounted filter medium.

The pressure in the purified water store tank is adjusted to 5 bar, resulting in a volume flow of 0.93 L/min in the nozzle. The angle between the surface of the mounted filter medium and the horizontal axis should be 45°. During the cleaning procedure, the mounting with the filter sample is permanently translationally moved through the nozzle flow for 90 sec. The wastewater is removed beneath the glass body into a liquid separator with a sterile filter designed for excess pressure. After cleaning, the cleaning room was disassembled and the demounted filter medium was put in the compartment dryer at 60 °C for 24 hours. Thus the cleaning procedure is finished and the third step, the preparation, can start.

To detect the degree of cleanability and therefore to verify the growth of microorganism, a special kind of nutrient medium, the Shapton & Hindes Agar (SHA), is used. This medium contains bromcresol purple as a pH sensitive dye. If spores remain on the surface or within the filter medium after cleaning, they will germinate during incubation, start to grow and ferment the glucose on the agar to acidic end points. These acidic metabolites reduce the pH of the agar and the bromcresol purple changes to yellow [10].

Prior to the analysis of such residues, molten Shapton & Hindes Agar has to be poured onto Petri dishes as a thin transparent layer and fully solidified. Then the side of the filter sample, which has been cleaned, will be carefully put on the surface of the solidified agar. Compared with the EHEDG test method it is impossible to pour over the filter medium with the molten agar, because the spores will be distributed all over the volume and an exact analysis will not be possible.

With this in mind, the filter sample was put on the solidified transparent agar surface without changing the conditions. Hence, after such a procedure the microorganism was located at its first place of growth. Afterwards the Petri dish was sealed up and incubated at 60 °C for 18 h. During the incubation process, a digital camera took photos every 1.0–1.5 h of the filter surface to document the progress of growth. These pictures were taken from below and exhibit the surface between the filter sample and the agar (see Fig. 5). Hereby the SHA was transparent. After incubation, the filter sample was examined for the presence of areas of yellow discoloration in the purple SHA.

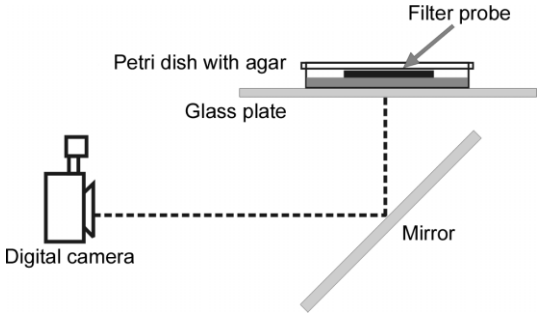

Figure 5. Recording scheme during the incubation process.

Analytical Methods

During the incubation process, the camera takes approx. twelve consecutive photos of the filter surface. With this means, a temporal resolution is practicable. Therefore not only the yellow areas at the end of incubation are detectable, but also the starting points and growth rates. Velocity and covered areas are important to compare the samples with each other.

To visualize the borderlines and determine the colored areas, the pictures where enhanced and processed by software (see Fig. 6).

A new parameter is needed for the comparison of the results of the different filter samples. This so called discoloration quotient Z_d was defined as follows:

$$Z_d = \frac{A_Y}{A_F} \quad (2)$$

A_Y describes the yellow colored area caused by the growth of microorganisms. A_F is the maximum surface (2124 mm^2) of the filter sample. Z_d values close to 1.0 indicate a complete spread of microorganisms all over the surface of the filter sample. In this case, the cleaning procedure has been insufficient

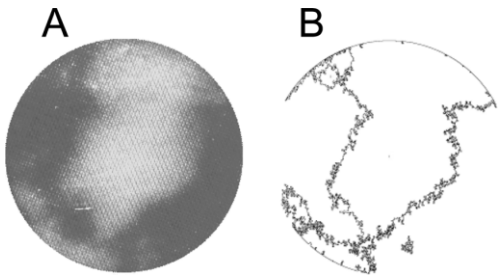

Figure 6. A) Picture edited with Adobe Photoshop®. (The light areas indicate the growth of microorganisms).
B) Detection of borderlines and area measuring with ImageJ 1.33u

and a low degree of cleanability was assumed. If $Z_d = 0$, no microorganism or residues were detectable on the filter surface, therefore the cleaning procedure has been adequate.

For a more exactly comparison of the filter samples, the discoloration quotient was handled in a time diagram. The gradient of the curve was then used to visualize the velocity of growth during the incubation and is defined as follows:

$$s = \frac{Z_t + \Delta t - Z_t}{\Delta t} \quad (3)$$

Whereas Δt expresses the time difference in-between two measurements (= in-between two digital pictures). Large values stand for an aggressive spread of organisms on the filter surface.

The entire classification of the different filters was achieved by the wet-bulb volume, discoloration quotient and the slope of the curve. These three parameters allow summing up a sequence for the cleaning of the five filter media tested.

3 Results

3.1 Modifications Compared with the EHEDG Test Method

Prior to obtaining reproducible results, the five different filter samples have been tested several times to find out the range of variation of parameters for the single steps of the test.

In the standardized EHEDG cleaning test method [10–11], soil consists of sour milk and *Bacillus stearothermophilus* var. *calidolactis* spores in a concentration of 10^5 spores/mL. Several different tests with this kind of contamination mixture proved its unsuitability for these new filter test methods regarding handling and analysis of the results. That is why the matrix of spores changed to purified water and the concentration had to decrease.

There are two major advantages considering the production and the handling of the new mixture. The first advantage is, that every laboratory produces its own purified water routinely and therefore it is always available. Sour milk, on the other hand, which is used in the current microbiological test methods, was prepared by adding a mesophilic starter culture to a suitable commercial milk volume. After that, the inoculated

milk is incubated at 30 °C for 24 h [10]. Consequently, the production of sour milk is much more costly than using purified water. Secondly, the handling during the contamination step of the new mixture is simpler, because the viscosity of water will not change if shear stress decreases. That was one of the major problems, which occurred on the surface of the filter medium. A visible filter cake of solidified milk was noticed after a contamination step with sour milk. It depends on the small flow velocity through the filter medium induced by the filter resistance and the pressure drop across the filter medium. The velocity has to be increased strongly to obtain a higher shear stress in order to avoid such cake formations. However, in this case the flow changed from laminar to turbulent conditions. Nonetheless since removing of residues creates more difficulties by such a pretreatment, the conditions should be adjusted to a laminar flow. Recently, a test with *Bacillus thuringiensis* on stainless steel surfaces provides information about the context between the flow conditions in a contamination step and the adhesion between the spores and the surface. These results show that for laminar flow, the spores were adhered much stronger on the surface than under turbulent conditions [11].

Another challenge was the determination of the right concentration of spores in the contamination mixture. The concentration in the current EHEDG test procedure was unsuitable in the new filter test method, since the amount of spores on the filter surface after the cleaning procedure was too high for an analysis. In order to remove the spores completely, it would have been necessary to extend the duration and pressure of the cleaning step appreciably. However, the limiting factor for that was the production of wastewater, which should be as little as possible. All wastewater must be autoclaved at 121 °C for 20 min prior to disposing into the sink.

Further on, the results of cleaning were affected by the angle between the mounting and the horizontal axis. Several tests have shown that an angle of approx. 45° was more successful for cleaning compared to a lower or higher one. In the purpose built nozzle cleaning system, a second feature was also improving the cleaning results. The mounted filter medium was moving through the jet flow permanently, resulting in a significant decrease in cleaning time. The intention for the adjustment of all these parameters during the cleaning step was the maximization of the efficiency of cleaning and the minimization of the amount of wastewater.

In the EHEDG test method, the cleanability of the components, which have been tested, were compared to the cleanability of a standardized reference pipe. After incubation, the test equipment is manually examined for the presence of areas of yellow discoloration in the purple agar using a comparison disc. The new filter test method utilized digital photographs and special computer software package, like Adobe Photoshop® and ImageJ, for the analysis.

In a recently designed recording unit, a digital camera will be installed in front of the incubator and pictures of the filter surface will be taken in variable intervals automatically. The results which are presented in this paper were obtained without this new unit. The pictures were taken manually, whereas the samples had to be placed on a template outside the incubator. Therefore, special care was taken to ensure that the orientation of the filter sample in the pictures was always the same

The method enables to analyze the results in different ways depending on the parameters of the software package. Therefore, it is possible to adjust several different levels for cleanability on demand. In the pharmaceutical industry not every component needs to reach the highest level of cleanability and lower levels are requested for cost reduction. With this analytical method, the degree of purity is adjustable. For the manufacturer of hygienic design equipment such information is quite valuable, since a validation process is both expensive and time consuming. If the component did not pass the test at a high level, then it will be downgraded to a lower one.

This new test method offers the opportunity to examine different filter media fully automatically regarding recording of growth and software analysis. Further on, a test process could be realized with the microorganism as a substitute for the real impurity. After testing and receiving satisfying results, the real validation process could start thereafter.

3.2 Results Regarding the Filter Samples Tested

For the classification of the different filter media, the wet-bulb volume was used. As mentioned above, these special parameters influenced the degree of cleanability. If the supporting layers were realized with huge apertures, the wet-bulb volume decreased extensively. Fig. 7 shows the summary of the results. It should be noted that the filter sample P5 with the biggest meshes of supporting layers gets the smallest wet-bulb volume. The samples P3 and P4 with smaller mesh sizes, achieve, on the other hand, the biggest one. It was assumed that the tendency for the remaining of spores or residues within the meshes would be higher in P3 and P4 compared to the other samples.

During the incubation step, the pictures were taken with a digital camera every 1.0–1.5 h. For instance in Fig. 8, the results of these photographs of P2 and P5 over the whole incubation distance are presented. It is noticeable that the spores were distributed over the surface of P2 but not of P5. Apparently, the cleaning procedure or the cleanability of the filter medium was more sufficient for P5 than it had been for P2. On the agar covering layer of P5 no growth was observed during the incubation time. It can be seen that the growth of organisms on P2 started in the middle of the sample, but there were isolated areas near the edges later on.

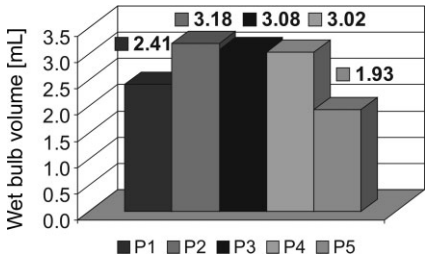

Figure 7. Wet-bulb volume for the five filter media tested.

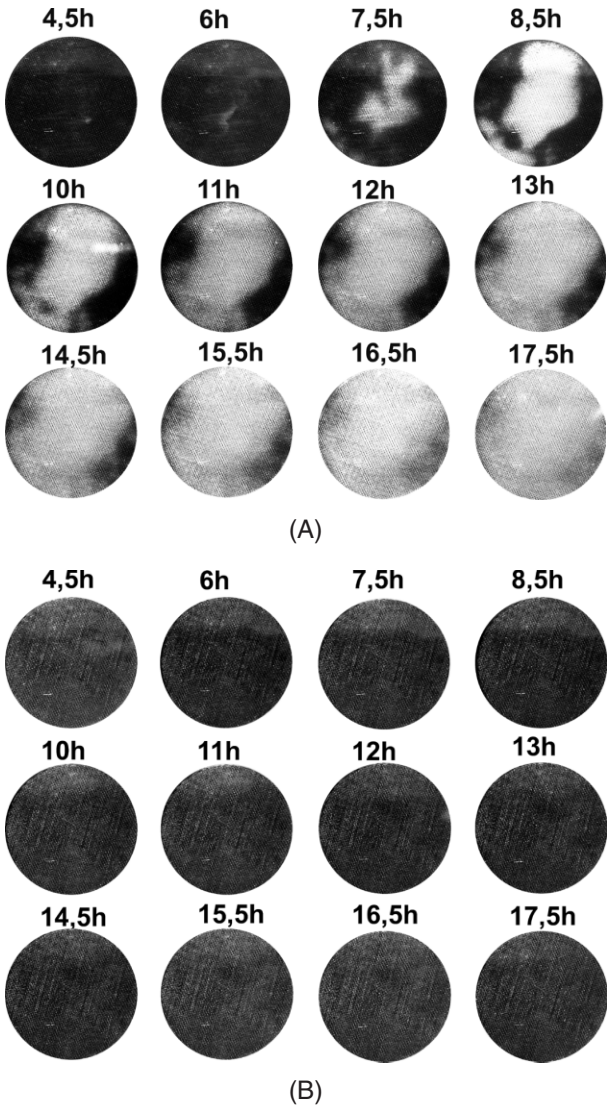

Figure 8. (A) Pictures of the cleaned surfaces lying on the agar und photographed from below. Medium P2 with starting growth in the middle of the sample.
(B) Pictures of the cleaned surfaces lying on the agar und photographed from below. Medium P5 without detecting any growth.

The graph in Fig. 9 shows the growth of yellow areas described by the discoloration quotient as a function of the incubation time for all the filter media tested. For comparing the single samples with each other, four criteria were important:
– existence of yellow areas,
– starting of growth,
– velocity of growth,
– yellow areas covering the sample at the end of the incubation time.

The existence of discolored areas proved the occurrence of residues on the surface. If there is no such visual effect on the filter sample, it can be assumed that the cleaning procedure was successful and the number of spores decreased significantly. This could only be found with P5, where no growth could be detected. As can be seen in Fig. 9, every other sample exhibited growth on the surface.

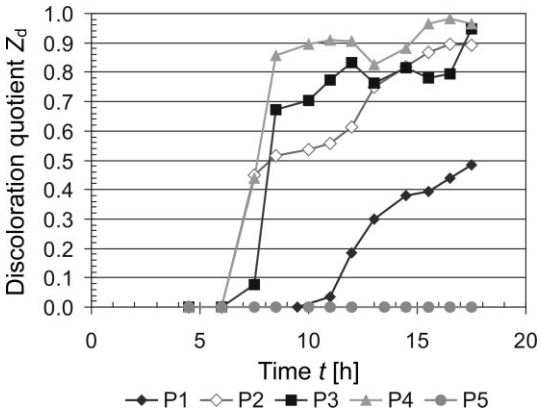

Figure 9. Course of the discoloration quotient as a function of time, without any detectable growth of microorganism on the surface of P5.

If spores are mainly present in the first layers nearby the agar surface, the time for a noticeable change decreased and the growth of the microorganisms was detected instantly. In this case, the cleanability of the tested filter medium is insufficient. P4 and P3 are the samples with earliest growth followed by P3 and P1. Observing the whole plots in the first hours, no growth is noticeable at all. After approx. 6 h, an aggressive growth process started mainly in the samples with big wet-bulb volumes (P2, P3 and P4). An exception is sample P1, where a noticeable growth was observed after 11 hours.

The velocity of growth, which is expressed by the gradient of the curve in Fig. 9, is the third evaluation criterion. Fig. 9 shows a narrowly constant gradient of the curve of sample P2 from the 7th hour to the end of the incubation time.

In the other cases, P4 and P3 exhibited a similar progress trend to P2. However, both curves are strongly rising after approx. 6.5 h of incubation, which means the yellow area grows more significantly. The maximum growth was reached after 7 to 8 hours of incubation and only a smooth descent is measurable thereafter. This result shows that much more spores are distributed over the surface of this sample as compared to sample P2. The growth of the organisms stops after approx. 8 hours, because of the inhibition of the spores among themselves and a lack of nutrition.

Another trend is observed in sample P1. Growth of *Bacillus* spores is first detected after 10 hours of incubation and starts very slowly only on a few points on the surface of the filter sample. Therefore, the number of spores is smaller compared to sample P2. The cleanability of P1 is much better than in P2, P3 and P4.

The last criterion is the yellow area at the end of the incubation process. It shows the growth of organisms and classified the samples depending on their cleanability. The bigger the yellow areas are the lower the degree of cleanability. Thus in this case, sample P5 obtains the best result, since no yellow areas are detectable.

Regarding the entire results of the test, the following tendencies became apparent concerning the cleanability: P5 > P1 > P2 > P3 > P4.

P5 is the sample with the smallest wet-bulb volume and shows the best cleanability. Considering the whole results, a correlation between the cleanability and the wet-bulb volume is proven. If the value of the wet-bulb volume increases, the cleanability will decrease.

4 Conclusions

In this study, a new microbiological test method is developed to validate the degree of cleanability of different filter samples. The test is based on the EHEDG test method for small sized components (e.g., valves, tubes).

A thermophilic test strain is used, as the impurity and the growth of this organism served as an indication of contamination. Therefore residues were detected regarding their locations on the surface of the filter media. The great advantage of this method is that even one spore could be detected during the incubation process. Hence, the test delivers results with rigorous accuracy and the components could reach a high hygienic level.

Currently the test is used to detect impurities of filter media. The intention for future studies is to use this technique in complex devices and furthermore to validate the degree of cleanability.

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