REPORT

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# Practical microbiological evaluation of air cleaner

## Summary

On behalf of Nanopas AB SP Food and Bioscience has performed a microbiological evaluation of an air cleaner. The air cleaner's ability to reduce growth of mold on bread and in milk as well as reducing growth of lactic acid bacteria in milk was examined in a test chamber. Compared to untreated control samples, bread inside the test chamber where the air cleaner had been operated had considerably less microbiological load up to six days after the start of the test. Milk samples inoculated with lactic acid bacteria, placed in a test chamber where the air cleaner was operated during five days, did not show any considerable growth reduction compared to untreated control samples. Milk samples inoculated with mold spores and placed in a test chamber during four days showed considerable lower levels of mold growth when treated with continuous air cleaning.

## Background

An air cleaner that is energy efficient and can effectively reduce the number of airborne mold spores, or retard their outgrowth to vegetative cells has a huge potential for implementation in the food industry and other sectors where good air quality is important. Low operating cost and minimal need for maintenance means that the equipment can be a relevant alternative to existing ventilation solutions, which are often based on the use of filters of various types that regularly need to be replaced for optimum performance. Previously conducted tests by SP and Nanopas indicate that the tested air cleaner can reduce the number of airborne particles in a room or a test chamber.

## Tested item

Nanopas AB, prototype model (Figure 1, A and B).

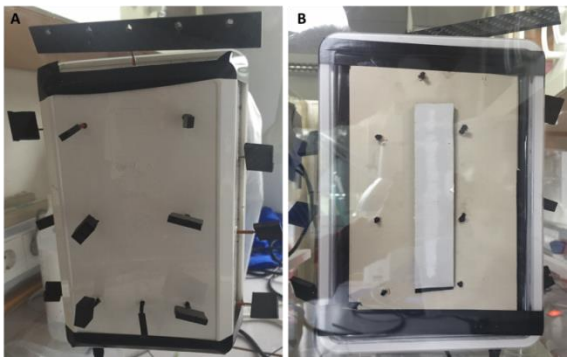


**Figure 1. A)** Air cleaner from above and **B)** from below.

## Place and date of testing

All tests summarized in this reported where conducted at SP Food and Bioscience microbiology laboratory during April to July 2016.

## Test methods and test procedure

All tests were performed inside a 40 cm x 40 cm x 45 cm (height) Plexiglas test chamber (Figure 2) kept at room temperature (21-23 °C). During the tests air humidity was continuously measured, and kept at an interval between 90 and 100 % using beakers filled with sterile water that were placed inside the test chamber. The air cleaner was mounted on one of the inside walls of the test chamber and allowed to operate for 1 hour prior to the start of each experiment.

### Bread tests

For the bread experiments bread not containing any preservatives where chosen in order to optimize the conditions for mold growth. The bread used for the tests where baked the same day as the experiment was conducted. The bread where placed at the bottom of the test chamber and the test initiated by spreading of mold spores (*Penicillium commune*) from a small plate placed in the upper part of the test chamber by means of pressurized air. To ensure that the spores where kept airborne, a small fan was used to move the air inside the test chamber during the first five minutes following the spreading of the spores. The outgrowth of spores to vegetative mold was monitored during six days and documented by taking photos of the bread. Control experiments where performed with spreading of mold spores in a test chamber without air cleaning. The exact concentration of spores that are spread is not possible to denote, but it is estimated to be at least 20 times the concentration normally present in ambient air. To illustrate this, control experiments where no spores were spread were also performed.

### Milk tests

In the milk tests lactic acid bacteria (*Lactobacillus plantarum*, ATCC 14917) and mold spores (*Rhizopus stolonifer*) were inoculated in beakers with milk placed in test chambers with and without continuous air cleaning. For the experiments low fat milk (0,5 % fat content was chosen). The tests were performed in triplicate. After five days the number of lactic acid bacteria/mold was analyzed by culturing on relevant growth plates, MRS (de Man, Rogosa, Sharp) and MEA (Malt Extract Agar) respectively. After 2-3 days incubation at the permissive temperature the number of lactic acid bacteria and mold were quantified in treated and untreated samples.

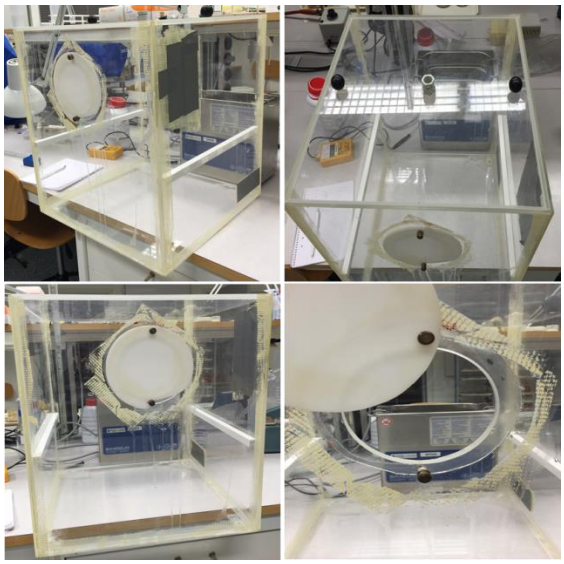

**Figure 2.** Test chamber used in the tests.

## Results

### Bread test with spreading of mold spores

Figure 3 shows the results from tests performed with bread placed in a test chamber during six days following spreading of *Penicillium commune* spores.

During the course of the test there is an observable progressive growth of mold in the control samples (upper panel). After six days all four bread in the test chamber without air cleaning display clearly visible growth of mold. In the test chamber with continuous air cleaning only one out of four bread shows clearly visible mold growth after six days.

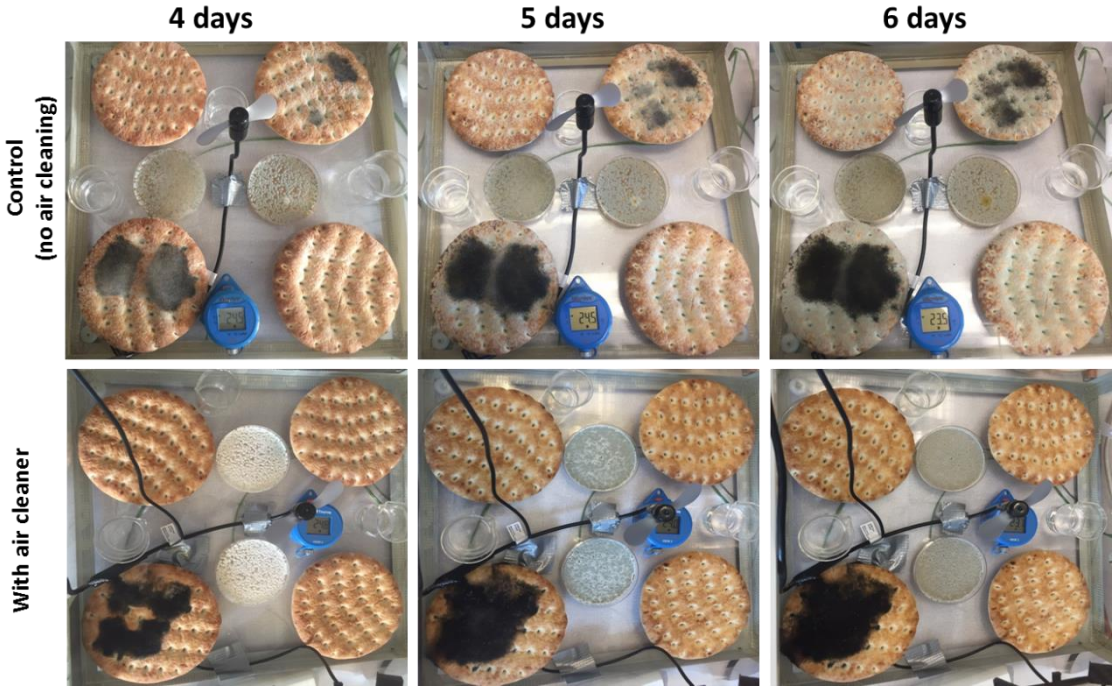


**Figure 3**. Bread placed in test chamber without air cleaning (upper panel) and with continuous air cleaning (lower panel) during six days following spreading of *Penicillium commune* spores.

**Bread test without spreading of mold spores (ambient air)**

Figure 4 shows the results from tests performed with bread placed in a test chamber during six days in ambient air (no spreading of mold spores).

Bread in test chamber with air cleaning (lower panel) have substantially lesser degree of visible growth of mold compared to the untreated control (upper panel).

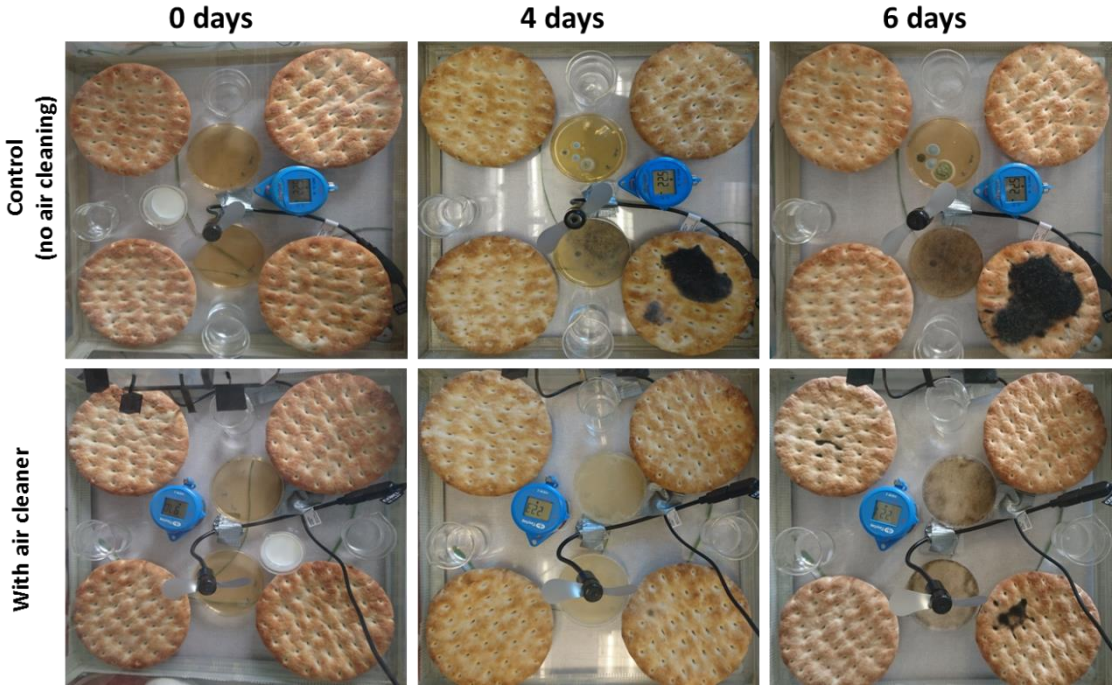


**Figure 4**. Bread placed in test chamber without air cleaning (upper panel) and with continuous air cleaning (lower panel) during six days in ambient air.

**Milk test with lactic acid bacteria**

Figures 5 and 6 show results from the test performed with beakers of low fat milk inoculated with the lactic acid bacteria *Lactobacillus plantarum* and placed in a test chamber during five days. No clearly measurable difference between samples treated with continuous air cleaning and the untreated control was observed when samples were taken and analyzed by culturing on MRS growth plates (Figure 5). The antimicrobial effect is apparently not possible to achieve in liquid during these testing conditions. However, when comparing samples with air cleaning (Figure 6, lower panel) and the untreated control (Figure 6, upper panel) it appears as though air cleaning is able to reduce the outgrowth of mold spores present in ambient air to vegetative cells. Vegetative mold cells are present both on MEA control plates and in the milk in the untreated control after five days. In one of the beakers with treated milk the milk appears to have separated into two distinguishable phases after five days (Figure 6, bottom right corner).

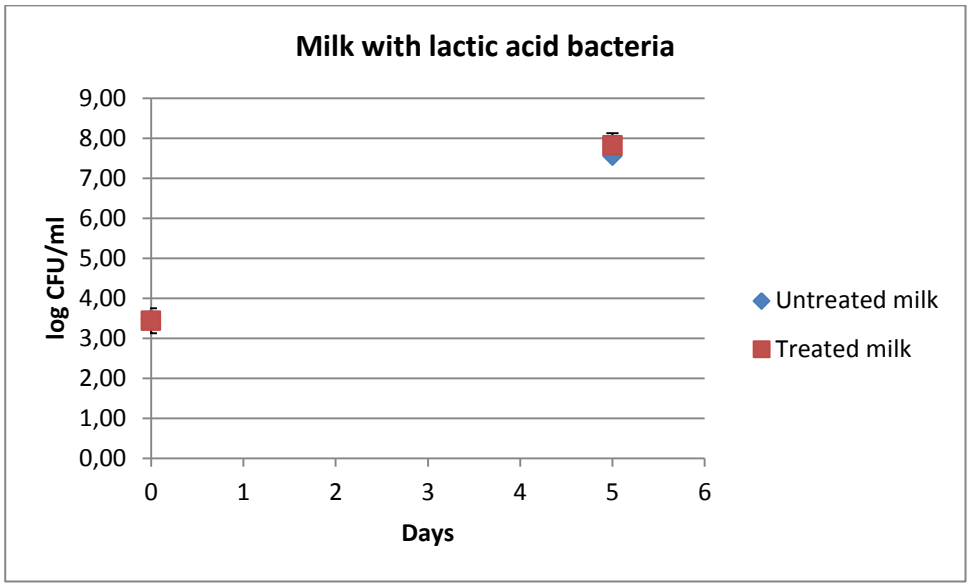


**Figure 5**. Growth of lactic acid bacteria (*Lactobacillus plantarum*) in milk placed in test chamber without air cleaning (blue) and with continuous air cleaning (red) during five days in ambient air. Standard deviation based on triplicate samples is indicated with error bars.

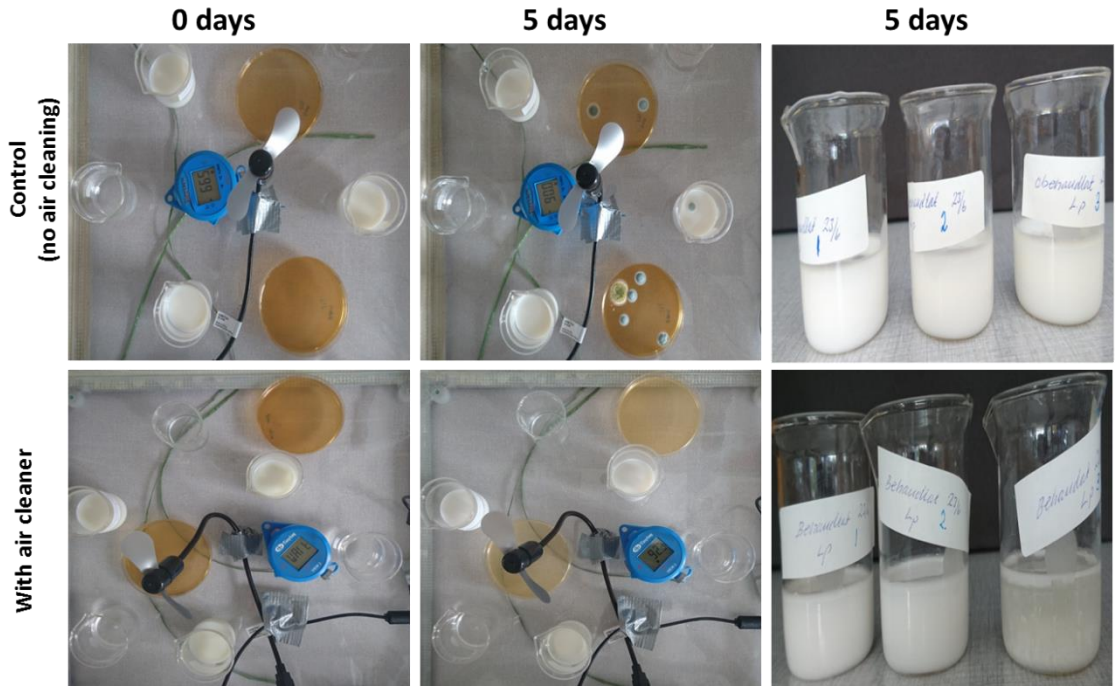


**Figure 6**. Beakers with milk inoculated with lactic acid bacteria (*Lactobacillus plantarum*) and placed in test chamber without air cleaning (upper panel) and with continuous air cleaning (lower panel) during five days.

### Milk test with mold spores

Figure 7 shows the result from the test performed with beakers of low fat milk inoculated with spores of the mold species *Rhizopus stolonifer* and placed in a test chamber during four days. There is a clear difference in visible growth of *R. stolonifer* between samples treated with continuous air cleaning (Figure 7, lower panel) and the untreated control (Figure 7, upper panel) on the surface of milk in beakers. As in previously conducted tests, it appears as though air cleaning is also able to reduce the outgrowth of mold spores present in ambient air to vegetative cells. This is reflected by the absence of mold growth on MEA control plates in the test chamber with continuous air cleaning (Figure 7, lower panel). No separation into different phases (liquid and fatty) was observed in the treated milk samples, but the surface of the milk was slightly dried and hardened compared to the untreated samples.

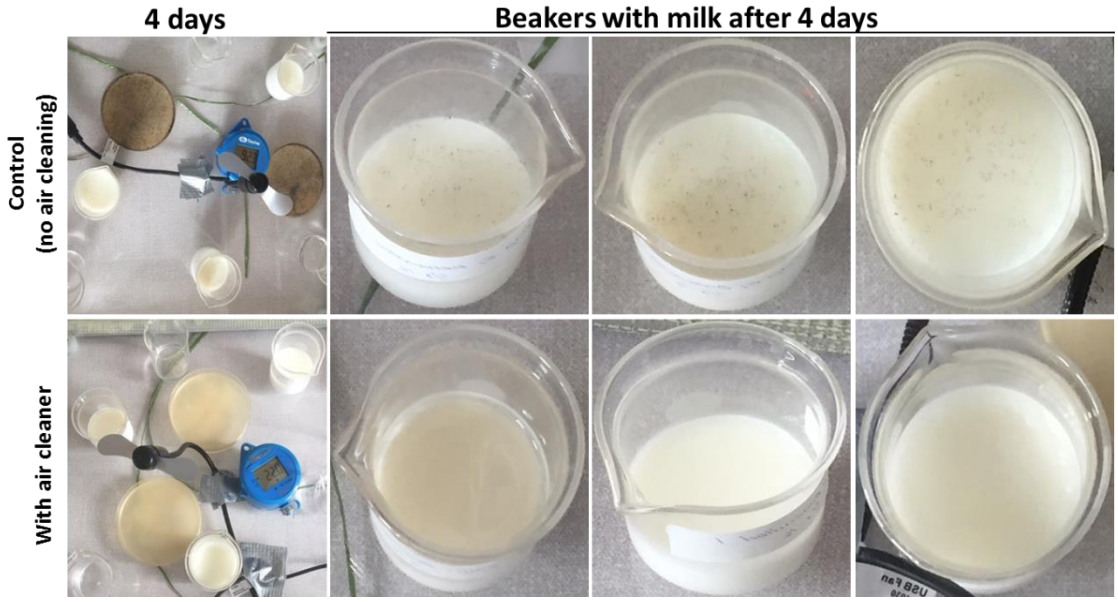


**Figure 7**. Beakers with milk inoculated with mold spores (*Rhizopus stolonifer*) and placed in test chamber without air cleaning (upper panel) and with continuous air cleaning (lower panel) during four days. Close-ups show presence/absence of growth in individual beakers. All milk samples were inoculated with the same initial concentration of mold spores, set to log 3 spores/ml.

## Conclusion, discussion and future perspectives

Initial tests (not included in this report) showed that mold spores of the species *Aspergillus brasiliensis* on MEA growth plates that had been treated with continuous air cleaning showed delayed sporulation, which is the reproductive phase in the molds life cycle. From the performed tests with bread it was evident that air cleaning also delays the outgrowth of mold spores of the species *Penicillium commune* to vegetative cells on the surface of bread. This was true for both conditions with excessive air concentration of airborne spores (Figure 3) and in ambient air (Figure 4).

In milk samples inoculated with lactic acid bacteria (*L. plantarum*) no reduction in growth was observed between treated and untreated samples (Figures 5 and 6). This is reflecting the ability of the current prototype of the air cleaner to reduce microbial growth in air but not in liquid. Also, even though lactic acid bacteria can grow in the presence of oxygen, they prefer to grow anaerobically (absence of air) or at lower concentrations of oxygen, which is the case in the milk suspension, but not on the surface of the milk.

In milk samples inoculated with mold spores (*R. stolonifer*) there was a clear difference in visible mold growth between treated and untreated samples on the surface of milk after four days. The mold spores undergo a change from the inert spore form to the vegetative form in the interface region between liquid and air at the surface of the milk, which results in growth in the untreated control samples (Figure 7, upper panel). However, during conditions with continuous air cleaning this growth is inhibited (Figure 7, lower panel).

The results obtained with Nanopas air cleaner are highly promising. Further tests can verify whether the apparatus is applicable for use in an industry setting to reduce or inhibit problems associated with growth of mold, yeast and bacteria both on food and on the surface of various materials. The established experimental set-up presented in this report is applicable to other types of fruits, vegetables or food products to show effect of air cleaning. It would for example be of interest to perform tests with apples or strawberries in order to evaluate if they can retain good quality for longer time with continuous air cleaning. By delaying the microbiologically induced spoilage of food stuff such as fruit and vegetables, substantial economic gains can be made by reducing food waste during transport and storage.

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**Food and Bioscience - Microbiology and Process Hygiene**

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