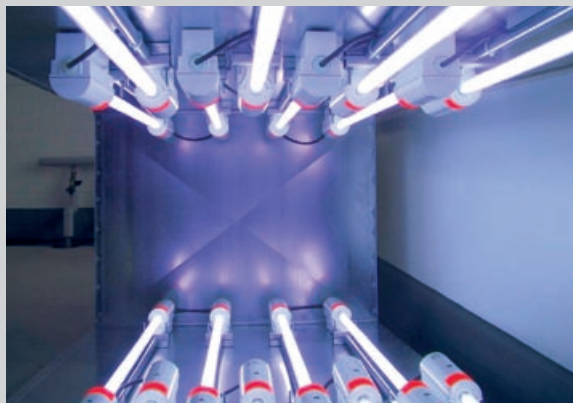




## UV-C TECHNOLOGY

Inactivation  
of bacteriophages  
in milk-processing  
plants





## Use of UV-C technology to inactivate bacteriophages in milk-proce

Our daily diet is enriched by a range of fermented products such as cheese, meat products, vegetables, bread and wine. Common to the production of these is the use of bacterial strains. These production methods would be inconceivable without the metabolic activity of the bacteria used in each process.

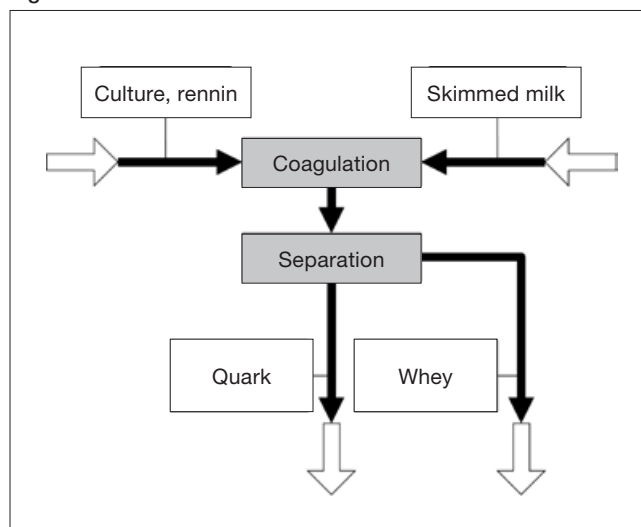
In 2000 and 2001 some 27 million tons of milk were turned into milk products in Germany alone. Of this around 750,000 tons were made into quark and cream cheese; this corresponds to an annual consumption of 9 kg per year per head of population (Federal Statistical Office).

Whereas in former times acidification was a result of the natural milk flora, today pure or mixed cultures are systematically added to the milk.

The most important feature of the lactic acid bacteria, that is the ability to form lactic acid from lactose, fulfils two functions. First, the shelf life of the milk products is increased through the reduction of the pH value without loss of nutritional physiological value. Second, lowering the pH value to below 4.6 to 4.8 causes precipitation of the milk casein, which has a positive effect on the consistency of many products.

**Figure 1** illustrates the production of quark. Pasteurised milk is mixed with lactic acid bacteria and rennin in rennin tanks. On coagulation of the milk the whey is separated from the product (separators). The use of starter cultures has had a considerable effect on improving the product quality. At the same time the demands made on the cultures have increased over time. Alongside the various *lactobacillus*, *leuconostoc* and *streptococcus* strains, *lactococcus* strains are also used for the fermentation of milk products. They are indispensable for the production of various cheese types, butter and quark in particular.

**Figure 1**



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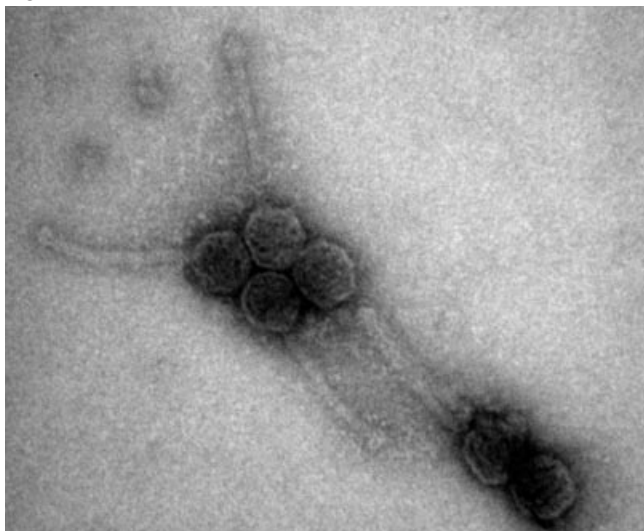
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40% of food consumed in Germany today is produced with the aid of starter cultures and/or enzymes. Some cultures also contain strains that convert citrate into odour and flavour components. Acidification problems in the production of dairy products can be due to various causes, for example bacterial contamination or the loss of various characteristics of the bacterial strains.

The largest economic loss to milk-processing companies (with 70 to 80% the main cause of all production problems) is due to bacteriophages (phages). Phages are viruses that infect bacteria. The infestation of a bacterial culture with phages can lead to the death of the bacterial cell and thus cause minor or total loss of production. Due to their small size, phages cannot be identified with the naked eye or in the light-optical microscope. **Figure 2** shows an image of a *lactococcus* phage taken using an electron microscope. They are smaller than 200 nm in size.

Figure 2



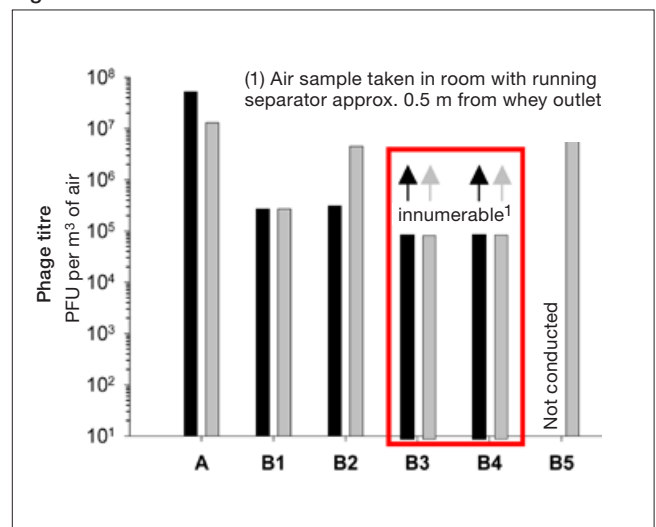
It has been known for over 70 years that the reason for loss of fermentation was the infection of the cultures with bacteriophages. The growth of starter cultures during a fermentation infected by bacteriophages can be severely slowed down ("slow vats") or not take place at all ("dead vats").

Possible causes of infection remain today insufficient heating or disinfection of the milk or the dissemination of the phages via the air.

The risk of a bacteriophage infection during fermentation is very high as most processes in milk-processing plants take place in semi-sterile environments. The problem is even greater today since milk fermentation often takes place on an industrial scale in 50,000-litre tanks and problems can mean significant economic loss.

Phages can be found not only in untreated raw milk, but also primarily in whey and can be distributed through the air over long periods.

Figure 3



The highest phage titres within a milk-processing plant with up to  $10^8$  phages per  $m^3$  of air were measured next to a running separator, **Figure 3**. It can therefore be assumed in the production areas that these separators that separate the whey from the product nebulise the phage-bearing whey and cause a permanent distribution of the aerosol within the plant. The air therefore forms a transport system for phages to a certain extent. The phage titre reduces with increasing distance from the separator.

### Objective

Phages are found in the entire production area. A primary, clear objective of hygiene monitoring must therefore be the protection of the starter culture from phage infections. Thus the plant-level input required in growing the live culture (starter) is considerable. Phages that find their

way into the culture before or during growth have an opportunity to grow to a technologically critical level at the culture preparation stage. Filters do not provide comprehensive protection as some phages are smaller than the standard pore sizes.

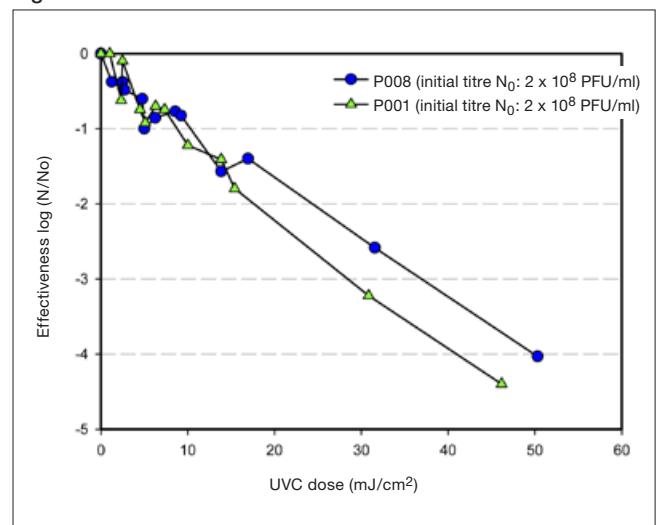
Further, the plant technology must not be left out of the equation. For example, in today's cheese-making plants CIP systems purify both whey-treatment systems (high phage risk) and also culture systems. If phages survive the cleaning and disinfection solutions, a recontamination in sensitive areas may occur.

Treating the air with UV-C radiation is an ideal method of keeping the room air largely phage-free and thus reducing the pressure on the starter culture. In this regard it is essential to retain an overview of the possibilities of phage reduction. For this reason tests to inactivate phages using UV radiation were carried out.

Figure 4



Figure 5



## Test procedure

A test environment was developed for the laboratory tests to enable phages in fluid media to be inactivated by UV-C radiation. An existing PCR cabin was converted to enable integrated UV-C tubes to be used. Slot-in compartments were created at defined distances to the UV-C tube that enabled UV-C intensities of  $0.6 \text{ mW/cm}^2$  to be achieved, **Figure 4**. In conjunction with the exposure time, UV-C doses of below 2 to above  $50 \text{ mJ/cm}^2$  were achieved.

The tests showed that various *lactococcus* phages (P001 and P008) reacted differently to UV-C radiation. A UV-C dose of  $30 \text{ mJ/cm}^2$  on the P008 phage caused a reduction of 2.5 log stages, and on P001 a reduction of over three log stages. A dose of up to  $50 \text{ mJ/cm}^2$  caused a reduction in both phage types of at least four log stages.

This means that the initial quantity of  $10^8$  phages per ml was reduced to just 104 phages after irradiation, or in other words, at  $50 \text{ mJ/cm}^2$  at least 99.99% of phages were inactivated. Tests on the *E. coli* phage MS-2 showed that at least  $119 \text{ mJ/cm}^2$  were required to achieve the same inactivation rate. At  $10 \text{ mJ/cm}^2$  our tests nonetheless enabled reduction rates of at least 90% to be achieved, **Figure 5**.

For use under practical conditions a test environment was designed and built, **Figure 6**. Settling stretches were fitted at the inlet and outlet areas of an existing UV-C recirculation disinfection module. These enable the phage content of the supply and exhaust air to be recorded separately as required using an airborne germ collection unit and a sensitive culture. This module is transportable and can be easily integrated into an existing production room.

## Outlook

The tests produced promising results and showed clearly that *lactococcus* phages could be significantly reduced using technically feasible UV-C systems. This technology could be expedient both in the growth of the cultures and also within the production plant. For example, it could be used to enclose the separators to largely eliminate what is by far the highest source of emissions. This solution is even more important if live culture tanks are not located in a separate room or rennin tanks, where milk is mixed with culture and rennin, are still of an open top design.

This technology could conceivably be adapted to similar areas. As well as all milk-processing plants, it could also be fitted in meat plants. Other phage sources such as saline baths could also be kept largely phage-free with this technology.

Figure 6





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