Separators from GEA Westfalia Separator for Milk Clarification and Bacteria Removal
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1. Introduction

Clarifiers from GEA Westfalia Separator Group and bacteria-removing separators are used in the dairy industry to improve milk quality. Centrifugal and/or membrane technology are used to separate impurities and bacteria from the milk.

Examples of undesired constituents in raw milk are particles of dirt, blood residues, udder cells and a great many different bacteria.

This technical documentation explains clarifying and bacteria removal efficiency in relation to the processes used in the field. Among other things, this clearly shows the composition of the phases discharged in batches when clarifiers and bacteria-removing separators are used, and the extent to which these phases can be recycled.
2. Milk Clarification

2.1 Introduction
The most important part of clarifying milk is the separation of non-milk solids (NMS). A distinction is generally made between the different methods used by processors to improve the quality of milk.

2.1.1 Clarifying milk using filters
This method of improving the quality of milk has been more or less abandoned these days for a variety of reasons.

One of its biggest drawbacks is the drop in flow rate over time because a thicker and thicker filter layer builds up. Running time is limited. The entire milk flow is passed through the filter layer. This allows bacteriological problems to arise due to entrainment, there is a risk of bacterial growth in the filter layer and thus reinfection of the milk. What is more, if there are cracks in the filter tissue, the clarifying effect is considerably reduced. Cleaning the filters after production is also an extremely laborious process.

The use of filters to improve milk quality should not, however, be confused with initial straining to separate "coarse" impurities such as foreign bodies, wood, cellulose, or packaging residues from returned milk. It is essential that the milk is strained before processing continues so as to prevent damage to sensitive parts of the line. Pore width may not exceed 0.2 mm.

2.1.2 Clarifying milk using the skimming separator
Independent of the separation of milk into skim milk and cream, every skimming centrifuge has a secondary effect, namely separation of solids from the milk. The separated solids are discharged in batches by means of partial ejections. The clarifying effect achieved with a milk separator is better and more stable than when filters are used.

However, the separation rate of solids is even higher in clarifiers especially designed for this purpose than it is in skimming separators.

2.1.3 Clarifying milk using the clarifier
Clarifiers are machines specifically designed for solids/liquid separation. The specific design of this machine enables it to achieve an optimum separation rate for impurities. We will go into the design differences in more detail at a later stage.

2.1.4 Composition of solids discharged by separators
Complex studies have been conducted to obtain precise information about the solids discharged by self-cleaning separators during milk clarification. Samples from different milk regions using separators of different sizes have been analysed, thus ensuring a representative cross-section.

Partial ejections were performed on all separators. The time between two consecutive partial ejections was selected so that the solids had a dry mass of 14 to 16 percent. This ensured that at each partial ejection, all the solids separated were discharged. The results of the study are shown in Fig. 1.

![Fig. 1 Analysis values for an ejection](chart)

- Water approx. 84 %
- Protein 6 – 8 %
- Lactose approx. 4.7 %
- NMS 1.5 – 3 %
- Fat 0.25 – 0.35 %
The values shown in Fig. 1 are based on the following further conditions:

- 0.05 – 0.1 percent by volume related to the quantity of raw milk fed in was discharged by partial ejection
- Separation temperature was between 45 and 55 °C

The significance of separation temperature will be explained in more detail later on in the documentation.

2.1.5 Proportion of non-milk solids in milk

The data available allow the proportion of NMS in the raw milk to be calculated, with NMS % (DM) being assumed to be equal to NMS % by vol. as an initial approximation.

\[
NMS_{max} = \frac{\text{proportion of ejection volume} \cdot \text{proportion of NMS in ejection volume}}{100}
\]

\[
NMS_{max} = \frac{0.1 \cdot 3}{100} = 0.003 \%
\]

\[
NMS_{min} = \frac{0.05 \cdot 1.5}{100} = 0.00075 \%
\]

2.1.6 Product losses when clarifying milk using separators

Product is lost as a result of spun-off solids being discharged from the separator bowl. An example calculation of product loss would be: quantity of milk fed in 25,000 kg/h, partial ejection every 30 min., partial ejection quantity 8 kg.

\[
\text{Product loss, absolute} = \frac{\text{ejection quantity kg/h} \cdot 100}{\text{feed quantity kg/h}}
\]

\[
\text{Product loss, absolute} = \frac{16 \text{ kg/h} \cdot 100}{25,000 \text{ kg/h}} = 0.064 \%
\]

2.1.7 Protein losses when cleaning milk using separators

The solids discharged contain protein, among other things. The absolute loss of protein from partial ejection can be calculated as follows:

\[
\text{Protein loss, absolute} = \frac{\text{ejection quantity (kg/h)} \cdot \text{protein content of ejection} (\%) \cdot 100}{\text{feed quantity to separator (kg/h)} \cdot \text{protein fed in} (\%)}
\]

\[
\text{Protein loss, absolute} = \frac{16 \text{ kg/h} \cdot 7 \% \cdot 100}{25,000 \text{ kg/h} \cdot 3.2 \%} = 0.14 \%
\]
The following factors are critical for the level of protein losses:

- The quantity of solids discharged in partial ejection and the time between two partial ejection cycles (ejection interval)
- The ratio between the quantity of solids discharged and raw milk fed in should be between 0.05 and 0.1 percent.
- Separation temperature should be no more than 55°C; a considerable rise in loss of protein is found above this.

A reduction in protein losses to 0.12 percent is considered perfectly feasible. The protein losses are illustrated in Fig. 2.

![Protein loss as a function of the quantity of solids discharged and temperature](image)

**Fig. 2** Protein loss as a function of the quantity of solids discharged and temperature

### 2.1.8 Temperatures when clarifying milk

A temperature either between 8°C and 15°C (storage temperature) or between 52°C and 58°C is recommended.

The recommendation of these limited temperature ranges is based on two pieces of information:

- Between 15°C and 35°C, there is an increased risk of damage to fat. This has been found as a result of the rise in free fat (FF) when the milk is under mechanical load (e.g. from pumps). Lipases are still active up to approx. 50°C.
- The temperature range from 30°C to 45°C represents an optimum for the growth of bacteria (even if these are present only in theory).
2.1.9 Reducing total bacteria count

More recent investigations on modern milk clarifiers have shown that compared to earlier statements, a significant proportion of the total germ count (TGC) is discharged with the solids.

As Fig. 3 demonstrates, however, this can only be achieved in milk cleaning at an appropriate product temperature.

![Reduction in total bacteria count (TBC) as a function of milk temperature](image)

**Fig. 3** Reduction in total bacteria count (TBC) as a function of milk temperature

2.1.10 Clarification effect when using skimming separators

In many areas of the dairy industry, it is customary for milk clarification to be combined with skimming. The current state of knowledge, however, allows a much greater clarification effect to be achieved with the use of clarifiers than is possible with skimming separators. Extensive in-house investigations have shown that only 30 to 50 percent of the NMS are separated from the milk in a skimming separator.
2.1.11 Separating somatic cells
A clear and easily proven indicator of the cleaning efficiency of a separator is the separation of somatic cells. Fig. 4 compares how a skimming separator and a clarifier remove these cells.

The relatively low separation effect in the skimming separator can be explained by the fact that the separation path for the solids in the disk stack of a skimming separator is much shorter than in the clarifier. Section 2.4.2 goes into more detail about the design differences between these separators.

![Fig. 4 Separation of somatic cells in the milk separator](Here the efficiency is shown for the specific somatic cell number of 430,000 cells/ml)

Efficiency $= \frac{SC_0 - SC_1}{SC_0} \cdot 100$ ($SC = $ somatic cells)

2.1.12 Separating listeria from raw milk
Listeria have a high affinity for leukocytes, in other words for a particular part of somatic cells. Some of the listeria trapped in leukocytes are resistant to heat, whereas free listeria can be killed off at pasteurization temperature.

To prevent a potential listeria problem, it therefore makes sense to separate the somatic cells in the raw milk as far as possible for two reasons:

- Leukocytes with "trapped" listeria are separated off
- If there are no leukocytes, listeria can furthermore not be trapped and are thus not resistant to heat. Any listeria which are present will be killed off during pasteurization.

This is why clarifying raw milk with clarifiers is always to be recommended.
2.1.13 Particular issues for clarifying milk
As an example of the many tasks performed by the milk clarification step, we will look here at the separation of 'pulverized' hairs from milk. In-house studies and studies at the University of Wisconsin resulted in up to seven different types of hair in approx. 70 percent of the cheese samples and about 40 percent of the fresh milk samples examined. It is advantageous if warm milk clarification is used in this instance, allowing all the particles of hair to be removed.
If the milk is clarified cold, on the other hand, only approx. 50 percent of these particles can be removed.

2.1.14 Summary
From the studies, we are able to draw the conclusion that warm clarification of milk, e.g. at 50 to 55°C, is preferable to cold clarification. This results from the fact that a significant proportion of the total germ count, but also particles such as pulverized straw or hair, are separated off. Separators have also proved the best alternative in the routine dairy tasks of clarifying from the points of view of cost, time and reliability. Separators are incorporated in the CIP cleaning circuit of the milk processing line, for example, so additional installations or detergents are not required specifically for the separators.

2.2 Clarifying raw milk cold – process technology
This method is frequently used in countries with a poor infrastructure, where the milk from small-scale producers is collected at central points. Centrifugal cleaning to improve quality is then performed before the milk is taken on for central processing at the dairy.

**Fig. 5 Method for clarifying raw milk cold**

1. Storage tank
2. Raw milk
3. Pump
4. Flowmeter
5. Constant pressure valve
6. Clarified milk
7. Solids tank
8. Solid
9. Clarifier
2.3 Clarifying raw milk warm – process technology
This method achieves the optimum clarification effect for the milk.

Fig. 6 Method for clarifying raw milk warm

1 Storage tank
2 Raw milk
3 Pump
4 Feed tank
5 Cleaned pasteurized milk
6 Heat exchanger
7 Flowmeter
8 Constant pressure valve
9 Clarifier
10 Solids tank
11 Solids pump
12 Solids
2.4 Clarifying milk – machinery

2.4.1 Method of operation of clarifiers
These days, GEA Westfalia Separator Group generally supplies clarifiers with a hydrosoft feed system. This system combines the benefits of softstream and a hydrohermetic feed.

• Adequate flow cross-sections mean low feed pressure
• Optimum design means great flexibility with regard to feed quantity
• No ribs in the feed chamber mean no shear forces – gentle product treatment
• Hydraulic seal means no air trapped in product

Figure 7:
The milk to be clarified flows through central feed tube (1) in the feed chamber which rotates at bowl speed. The feed to disc stack (5) is effected by bores in the base of the distributor.

The cleaned milk flows inwards and arrives in centripetal pump chamber (3). The milk is taken out of the rotating separator bowl under pressure and without foam by stationary centripetal pump (2). The solids slide outwards and accumulate in double cone-shaped solids chamber (6). A hydraulic system discharges the solids from the bowl at intervals which can be selected. Ejection is performed at full bowl speed.

Fig. 7 Bowl cross-section of a clarifier

1 Feed tube
2 Centripetal pump
3 Centripetal pump chamber
4 Disc stack
5 Feed to disc stack
6 Solids chamber
7 Discharge, clarified milk
**2.4.2 Processes in the disc interspace**

The large number of individual separation chambers arranged in parallel between the discs divides the milk stream into many thin layers. This minimizes the sedimentation route. A solids particle is considered separated once it has reached the bottom disc surface of the top disc. Flow speed is low here. The particle is no longer entrained with the flow, but slides outwards under the influence of centrifugal force. At the end of the disc, it leaves the separation chamber.

The smallest particle which can still be separated is separated on the separation route (I–II). The diameter of this type of particle is called the limit particle diameter. Figures 7 and 8 show individual separation chambers.

**2.4.3 Bowl ejections**

In addition to providing adjustable partial ejections, GEA Westfalia Separator Group ejection system also allows the bowl to be emptied completely. Depending on milk quality, partial ejections discharge a certain quantity of solids from the solids chamber at adjustable intervals. The feed to the separator remains open. Partial ejections discharge the incoming solids load from the bowl.

With total ejections, the complete bowl contents are discharged. The feed to the separator has to be interrupted briefly to do this. Total ejections clean all the surfaces inside the bowl due to the very high flow velocities. This is particularly important for chemical CIP of the line and separator. Total ejections of a separator during CIP guarantee optimum results.

As already mentioned in the section “Separating somatic cells”, the clarifying action of skimming separators is less than that of clarifiers. The reason for this is essentially the shorter separation paths. The separation path (I – II) of the skimming separator responsible for the separation of solids (Fig. 8) is generally only \( \frac{1}{4} \) as long as the separation route on the clarifier (Fig. 7). The cream which flows off accordingly still contains a proportion of non-milk solids (NMS). When the cream is mixed back in with the skim milk, this proportion of NMS returns to the milk.
2.5 Machine types
The following table lists current machine types and capacities of clarifiers for clarifying milk.

<table>
<thead>
<tr>
<th>Separator</th>
<th>Nominal capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEA Westfalia Separator eco clean</td>
<td>15,000 l/h</td>
</tr>
<tr>
<td>MSE 100-06-177</td>
<td>30,000 l/h</td>
</tr>
<tr>
<td>MSE 200-06-777</td>
<td>40,000 l/h</td>
</tr>
<tr>
<td>MSE 250-06-777</td>
<td>50,000 l/h</td>
</tr>
<tr>
<td>MSE 350-06-777</td>
<td>70,000 l/h</td>
</tr>
</tbody>
</table>

If products other than raw milk are clarified, enquire about the corresponding capacities.

2.6 Methods of testing clarification efficiency
The following method is suitable for determining the degree of purity of the milk.

Fig. 9 shows the equipment for a method (Funke Gerber) with three purity grades in accordance with the German standard.

0.5 l of milk are passed through a suitable cotton wool or fabric filter. Any particles of dirt are retained by the filter. The pattern of dirt is assessed and rated after the filter has dried.

Another method of assessing clarification efficiency is to measure the content of somatic cells (Fig. 10).
3. Bacteria Removal from Milk

3.1 General
The first attempts at removing bacteria from milk by centrifuge go back to the 1950s. However, it was not until the 1970s that bacteria were successfully removed from cheese milk on an industrial scale. In the 1980s, this technology finally experienced a breakthrough due to the development of bacteria-removing separators with a high degree of separation at simultaneous hourly outputs of up to 25,000 l/h. In recent years, the use of bacteria-removing separators has finally expanded successfully into other areas of milk processing. In addition to centrifugal removal of bacteria, filtration using membrane technology is also performed. In both methods, impurities and undesired germs or bacteria are separated from the milk. When milk is temperature-treated to inactivate bacteria and spores, undesired side effects such as changes in flavour may occur. However, methods such as irradiation with UV light or high-pressure technology do not currently play a role in the dairy industry.

3.1.1 Reasons for bacteria removal from milk
A number of objectives are pursued in removing bacteria from milk. In milk processing, for example, spore-formers can cause considerable problems. In the production of fresh milk, aerobic spore-formers (Bacillus cereus) impair shelf life as a result of sweet clotting.

In the production of milk powder, especially “low-heat” products, aerobic and anaerobic spore-formers (Bacillus cereus, Clostridium perfringens) lead to the product spoiling.

Under certain conditions, the removal of bacteria secures shelf life in soft cheese products – for example, in cases where the so-called ascospores of the moulds Byssoschlamys nivea or Byssoschlamys fulva have a negative impact on quality.

In whey processing, removal of bacteria makes particular sense when serum proteins are to be obtained from the clarified skimmed whey in concentrated form (WPC whey protein concentrate) by means of ultrafiltration. The long dwell time of the product in the filtration unit, some of that time spent at optimum incubation temperatures, leads to vigorous bacterial growth. According to the information available to us, there exist quality standards which stipulate that, for example, the content of anaerobic spores in 80 percent WPC may not exceed maximum five spores per gram of powder. This suggests that centrifugal removal of bacteria is the solution to improving quality.

Skim milk can also be treated by bacteria-removing separators before being processed into high-quality casein/caseinate so that it is of perfect bacteriological quality. Lactate-fermenting anaerobic spore-formers which are not killed off by normal milk heating can lead to butyric acid fermentation in the production of cheese. Greater attention is therefore paid to spore-formers of the genus Clostridium tyrobutyricum which cause late blowing in cheese. Lactobacilli also have to be removed in the production of raw milk cheese. As the milk is not heated above 50 °C at any point in the entire process, the lactobacilli which have not been killed off would lead to faults in the cheese.

3.1.2 Technical principles
Fig. 11 shows by way of example the route taken by Clostridium tyrobutyricum and the locations where it proliferates en route to the end product. Fig. 12 shows an example of the distribution of the Clostridia in raw milk and the corresponding metabolic properties.
**Route taken by Clostridium tyrobutyricum**

<table>
<thead>
<tr>
<th>Area</th>
<th>Preventing the growth of bacteria</th>
<th>Metabolic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Chemically: Formic acid, propionic acid etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biologically: Wilt, maize, vaccinate</td>
<td></td>
</tr>
<tr>
<td>Silage*</td>
<td>Milking hygiene</td>
<td></td>
</tr>
<tr>
<td>Rumen, faeces, raw milk</td>
<td>Physically: Separator</td>
<td>Dairy, cheese-maker</td>
</tr>
<tr>
<td>Vat milk</td>
<td>Chemically: Peroxide catalase</td>
<td></td>
</tr>
<tr>
<td>Cheese*</td>
<td>Chemically: Nitrates, lysozyme, nisin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Technically: pH, salt, temperature</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 11** Route and locations for the growth of Clostridium tyrobutyricum

* Locations for the growth of Clostridium tyrobutyricum

---

<table>
<thead>
<tr>
<th>No.</th>
<th>Fraction</th>
<th>Clostridium species</th>
<th>Metabolic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35 %</td>
<td>Clostridium sporengens</td>
<td>Decomposes protein (proteolytic)</td>
</tr>
<tr>
<td>2</td>
<td>12 %</td>
<td>Clostridium perfringens</td>
<td>Decomposes protein (proteolytic)</td>
</tr>
<tr>
<td>3</td>
<td>11 %</td>
<td>Clostridium butyricum</td>
<td>Decomposes lactose (lactolytic)</td>
</tr>
<tr>
<td>4</td>
<td>8 %</td>
<td>Clostridium tyrobutyricum</td>
<td>Ferments lactate (salt of lactic acid)</td>
</tr>
<tr>
<td>5</td>
<td>6 %</td>
<td>Clostridium beijerinckii</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7 %</td>
<td>Clostridium tetanomorphum</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4 %</td>
<td>Clostridium pasteurianum</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4 %</td>
<td>Clostridium tertium</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2 %</td>
<td>Clostridium novyi</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>≈11 %</td>
<td>Unclassifiable</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 12** Distribution of Clostridia in raw milk and their metabolic properties
The occurrence of individual strains in relation to the critical months of a year is shown in Fig. 13. These values are likely to apply to many milk catchment areas for dairies.

There are exceptions, however. In these cases, the total number of anaerobic and aerobic spores corresponds approximately to that in Fig. 13, but the ratio of anaerobic to aerobic spores is much more unfavourable. In Austria, for example, up to 50 percent of anaerobic spores were detected in the total spore quantity. In a North German dairy, up to 15 percent were found. In contrast to these significant deviations, a proportion of 2 to 5 percent of anaerobic spores was generally found in other studies.

An important factor in determining spores in milk for bacteria removal or from which bacteria have already been removed is the use of suitable test methods. When GEA Westfalia Separator Group quotes values, these are based on the test methods defined in this brochure (see chapter 3.9.1).

A large number of tests has now been carried out. The values determined result in a stable picture of spore content before and after removal of bacteria, allowing us also to make validated statements about bacterial clarification effect.

**Fig. 13** Number of spores and lactobacilli in raw milk
3.1.3 Effectiveness of bacteria-removing separators

The results of more recent tests, including some by independent institutes in cheese-making factories, are reproduced in Fig. 14.

Bacteria removal temperature

This should be between 55 °C and 62 °C. In this range, milk viscosity is relatively low. According to Stokes’ law, the sedimentation velocity of the bacteria to be separated off is higher than at lower temperatures. At higher temperatures, however, there is a risk of damage to protein.

Stokes’ law says:

\[
v = \frac{D^2 \cdot \Delta \rho \cdot g}{18 \cdot \eta}
\]

\(v\) = sedimentation velocity (m/s)
\(D\) = diameter of bacteria (m)
\(\Delta \rho\) = difference in densities of milk and bacteria (kg/m³)
\(\eta\) = dynamic viscosity of the milk (kg/ms)
\(g\) = gravitational acceleration (m/s²)

This equation clearly shows that sedimentation velocity “v” on the bacteria-removing separator is proportional to the square of the diameter of the bacteria, to the difference in density between milk and bacteria and to the acceleration due to gravity. It is inversely proportional to milk viscosity.

Separator feed capacity

Exceeding nominal capacity on the one hand results in a considerable reduction in bacteria-removing efficiency. Undershooting nominal capacity, on the other hand, only achieves a limited increase in efficiency.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Sample name</th>
<th>Temperature</th>
<th>Output</th>
<th>Quantity of concentrate</th>
<th>Ejection time</th>
<th>Anaerobic spores (Clostridia/10 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>Z 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 ml</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 1.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 1.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 2.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 2.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 3.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 3.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 4.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 4.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Tank sample</td>
<td>4T 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 ml</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 5.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 5.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 1</td>
<td>A 6.0</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1300 l/h</td>
<td>3600 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Process 2</td>
<td>A 6.1</td>
<td>50 °C</td>
<td>48,000 l/h</td>
<td>1400 l/h</td>
<td>3900 sec.</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Tank sample</td>
<td>4T 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 ml</td>
</tr>
</tbody>
</table>

Fig. 14 Results of double removal of bacteria using CSE 500-01-777 separators

Process 1: Bacterially clarified milk after the first machine
Process 2: Bacterially clarified milk after the second machine
3.1.4 Protein balance in bacteria-removing separators

Fig. 15 shows the product flows in the bacteria-removing separator.

The concentrate which continuously forms (entrained liquid) can be returned to the feed on bacteria-removing separators from GEA Westfalia Separator Group, or routed off for use elsewhere. The effect of bacteria removal is not influenced by the return of the concentrate.

Fig. 16 shows a table with product flows in the bacteria-removing separator and their protein content.

The protein content may deviate according to region and season. The mechanical differences of the GEA Westfalia Separator proplus design will be explained in a later section.

3.1.5 Treating concentrate

In addition to bacteria, continuous and batch-wise concentrate in the bacteria-removing separator contains protein and other valuable constituents. Depending on process management and regionally applicable regulations, the concentrates can be returned completely or partly to the milk following the appropriate treatment or processed further elsewhere. The batch-wise phase (ejections of the bacteria-removing separator) contains practically no non-milk solids if it was previously clarified by centrifugation or fully or partly skimmed by a separator. A temperature treatment (sterilization) is required to kill off the bacteria. This can be performed by UHT equipment, for example, which has been specifically adapted for the task. Direct steam injection or fine atomization in a steam chamber are possible methods of sterilizing the concentrate.

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>Feed</th>
<th>Discharge of bacterially clarified milk</th>
<th>Discharge, concentrate</th>
<th>Discharge, ejections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With concentrate recirculation</td>
<td>Without concentrate recirculation</td>
<td>Standard separator proplus separator</td>
</tr>
<tr>
<td>Quantity</td>
<td>50,000 l/h</td>
<td>49,960 l/h</td>
<td>48,460 l/h</td>
<td>1500 l/h</td>
</tr>
<tr>
<td>Fraction</td>
<td>100 %</td>
<td>99.92 %</td>
<td>96.92 %</td>
<td>3.00 %</td>
</tr>
<tr>
<td>Protein content</td>
<td>3.40 %</td>
<td>3.396 %</td>
<td>3.387 %</td>
<td>3.70 %</td>
</tr>
<tr>
<td>Protein units/h</td>
<td>1700</td>
<td>1696.80</td>
<td>1641.30</td>
<td>1698.80</td>
</tr>
</tbody>
</table>

Fig. 16 Product flows in bacteria-removing separator and protein contents
3.2 Bacteria removal from fresh milk – process technology

In this method, the separation of Bacillus cereus is of particular interest. This germ is heat-resistant and thus still active after pasteurization, so sweet clotting of milk can be the result. The specific weight and size of this bacillus make centrifugal separation difficult. Adapting separator feed capacity to the specific conditions, however, allows bacterial clarification efficiency of over 90 percent to be achieved.

In studies of pasteurized milk, orders of magnitude of 300 spores per litre were found for B. cereus. At a storage temperature from 8 to 10 °C and a generation time of about 6 hours, the bacillus multiplies from 1 spore per ml to over 107 spores per ml in around 6 days. This results in sweet clotting. This suggests that at a level of less than 1 spore per ml (e.g. at 1 spore per litre), shelf life can be extended by 10 generations, corresponding to around 2.5 days. Lower storage temperatures of the bacterially clarified and pasteurized milk (e.g. 4 to 6 °C) can extend the shelf life by up to 10 days.

Centrifugal removal of bacteria enables spores to be reduced by a factor of more than ten, corresponding to more than 3.5 generations.

Reduction in total bacteria count is often considered in assessing the removal of bacteria from fresh milk. However, it should be noted that the generally unknown distribution of flora across the various bacterial strains present can have a considerable influence on separation rate. One reason is the fact that the occurrence of small, lightweight bacteria may be comparatively low on one occasion and comparatively high on another.

Figure 17 shows the results of recent years which gives a relatively good picture of the range of separating efficiency (related to total bacteria count) which can normally be achieved.

\[
\text{Efficiency} = \frac{\text{TBC}_0 - \text{TBC}_1}{\text{TBC}_0} \times 100
\]

*Fig. 17 Separation based on total bacterial count in modern bacteria removing centrifuges (Here the efficiency is shown for the specific total bacterial count of 400,000 cells/ml)*
3.2.1 Bacteria removal from fresh milk – Stage 1

In this process variant, the entire milk stream has bacteria removed at 55 °C and is then fed directly to the skimming separator. In the separator, the milk is separated into skim milk and cream. The cream is then pasteurized and the fraction required to standardize the milk is diluted to a fat content of approx. 15 percent and homogenized. The cream is then mixed with the skim milk in the standardizer. The standardized fresh milk thus obtained is finally pasteurized and cooled. Both the fresh milk and the excess cream have had bacteria removed.
3.2.2 Bacteria removal from skim milk

When cream concentration at the skimming separator is set to 43 percent or higher, a high percentage of the spores remains in the skim milk. The reason for this is the significant difference in specific densities between the spores and high-fat cream. In this case, only the skim milk has the bacteria removed as shown in Fig. 20.

Fig. 20 Bacteria removal from skim milk

1 Bacterially clarified and standardized fresh milk
2 Excess cream
3 Raw milk
4 Bacteria-removing separator
5 Skimming separator
6 Standardizer
7 Homogenizer
3.3 Premium milk with a longer shelf life with the GEA Westfalia Separator prolong process

The production of premium milk with a longer shelf life is a question of freshness, naturalness, taste, vitamin content, and the number of actually necessary shelf life days.

**Freshness and quality indicators are advantages of prolong**

The content of ß-lactoglobulin on the one hand and lactulose on the other are common parameters for the milk quality and heat indicators. The content of ß-lactoglobulin in raw milk is approx. 3500 mg/l. The greater the extent to which milk protein is denatured by means of heat treatment, the greater is the extent to which this value declines. In the case of fresh milk, it amounts to 3000 mg/l in conjunction with pasteurization; in the case of micro-filtered milk, the figure falls to approx. 2500. In the case of milk subject to high heat treatment, it falls further to 1000 to 1600 mg/l, and may even be lower than 1000 mg/l in conjunction with indirect heating. By way of comparison, the indicator in conjunction with the prolong process remains at the level of fresh milk of approx. 3000 mg/l.

On the other hand, lactulose is not present in raw milk. It is a by-product of the chemical reaction of a heat treatment. The intensity of heat treatment is directly related to the quantity of lactulose to be found in the milk. In the case of pasteurized fresh milk, lactulose attains a value of 10 mg/kg; in the case of filtered milk, this figure is 17, and in the case of UHT milk, the figure is between 25 and (in conjunction with indirect heating) 32 mg/kg. With the prolong process, this factor is also identical to the fresh milk factor of 10 mg/kg. Both indicators for freshness and quality thus clearly underline the benefits of the prolong process.

**Two bacteria removing separators connected in series**

For this purpose, two bacteria removing separators connected in sequence are generally used directly upstream of the skimming separator in order to achieve a high degree of reliability with regard to removing the spores. This ensures that bacteria are genuinely removed from the entire quantity of raw milk, including the cream. The milk is then treated in the skimming separator with fat content adjustment and short-time heating.

An attractive economic aspect is that the separators can be used for other duties in the dairy, for example cheese production. An additional major benefit for dairies is also that bacteria removing centrifuges can be integrated in existing pasteurizing lines with minimal technical input.
Fig. 21 GEA Westfalia Separator prolong process

1. Pasteurized, standardized milk
2. Flow diverting valve
3. Holding tube
4. Heat exchanger
5. Hot water in/out
6. Booster pump
7. Raw milk in
8. Balance tank
9. Ice water
10. GEA Westfalia Separator standomat MC
11. Ice water
12. Cream cooler
13. Bacterial removal separator I
14. Bacterial removal separator II
15. Skimming separator
16. Surplus cream, cooled
17. Product pump
18. Homogenizer
3.3 ESL milk – process technology

In addition to “pasteurized fresh milk” and “long-life milk”, both of which have been on the fresh milk market for a long time, another kind of milk has come onto the market in the past few years, so-called “ESL fresh milk”. ESL is short for extended shelf life. Like pasteurized milk, ESL milk has to be kept in a cool chain to prevent it spoiling. Compared to long-life milk, there are fewer changes in the flavour of ESL milk, its “fresh character” being retained. Measures which lead to an extended shelf life in ESL milk are:

- Greater reduction in germ count compared to normal pasteurization
- Avoidance of recontamination following pasteurization.

One of the technical solutions for a drastic reduction in germ count is microfiltration.

Arrangement of a line to produce ESL milk using a microfiltration unit

Fig. 22 shows the arrangement of a line of this kind. The milk is heated up to 55 °C, polished in the bacteria-removing separator and separated into skim milk and cream in the skimming separator. Bacteria are then removed from the skim milk during microfiltration and the cream is subjected to UHT. A standardizer divides the flow of cream into excess cream and cream to standardize the milk. More or less heated cream is returned to the skim milk depending on the fat content the standardized ESL milk is supposed to have. Before remixing, this cream is diluted with skim milk and subjected to partial stream homogenization. After skim milk with the bacteria removed has been mixed together with UHT-treated homogenized cream, the standardized milk is pasteurized and cooled. The bacteria-removing separator in the first stage means that an optimum production time is achieved for the line downstream.

![Fig. 22 ESL milk line with microfiltration](image)
3.5 Bacteria removal from cheese milk – process technology

Increasing use of silage as feed in agriculture reduces milk quality in terms of germ loading, particularly by spore-formers. These spore-formers cannot be eliminated adequately by a temperature treatment, which is why removal of bacteria by centrifuge became more and more established in the past. This enabled quality problems, particularly late blowing, to be avoided.

Cheese-makers frequently standardize the milk (protein and fat content are adjusted) in tanks. The adjusted milk is warmed up and, depending on the cheese production process, pasteurized or if this has already happened, heat-treated. In these cases, the bacteria are removed from the milk immediately before the cheese vats are filled. Figure 23 shows a diagram of a cheese milk line with a bacteria-removing separator.
3.5.1 Double bacteria removal
If a single-stage bacteria removal process is not adequate to produce cheese without the addition of nitrate, for example, it is possible to arrange two bacteria-removing separators in series. In this arrangement, the second separator acts as a polisher and ensures low germ values in vat milk under all operating conditions in the production line. The results achieved are shown in Fig. 14.
Fig. 24 shows an example of an installation for double bacteria removal. The continuous concentrate can optionally be returned to the feeds or routed away for sterilization. There is also the option of merging all the concentrates produced both continuously and batch-wise (ejections) and of routing them for further processing.

3.5.2 Variable bacteria removal, example: cheese milk
With some types of cheese, the almost complete separation of all the germ flora has had a negative effect and this had to be counteracted by increased addition of culture or addition of raw milk to the milk with bacteria removed.
A concept for variable bacteria removal was worked out to solve this problem in collaboration with the regional dairy teaching and research institute in Wangen.
The aim of the development was to adapt the effect of bacteria removal to the requirements of the vat milk as a function of raw milk quality. To this end, trial cheeses were produced in the research institute’s technical centre by varying the speed of a bacteria-removing separator.

In Fig. 25, it can be clearly seen how both the propionic acid bacteria (Propioni) and the lactobacilli (at incubation temperatures of 30 °C and 45 °C) drop dramatically when the speed of the bacteria-removing separator is increased. The separation of Clostridia continued to be optimum even at reduced speeds because of its higher density. A lower number of the lactobacilli present in the vat milk causes the formation of lactate from lactose to slow down. This means that the propionic acid and butyric acid fermentation process in the cheese is also slowed down, which results in fewer holes forming and the flavour of the cheese being impaired.

![Fig. 25 Dependence of residual germ content on bowl speed](image)

- Lactobacilli 30 °C
- Lactobacilli 45 °C
- Propioni
The excessive removal of the propionic acid bacteria due to excessive removal of bacteria from the vat milk likewise leads to reduced propionic acid fermentation. In the trials, both types of bacteria removal at maximum efficiency of the bacteria-removing separator led to poor hole formation and to a slight deterioration in flavour. Reducing the bacteria removal efficiency of the separator by reducing speed, on the other hand, considerably improved both hole formation and the flavour and structure of the cheese.

**Conclusion**

Trials conducted by GEA Westfalia Separator Group in collaboration with the regional dairy teaching and research institute in Wangen showed that varying the speed of a bacteria-removing separator makes it possible to adapt bacteria removal to guarantee cheese quality whilst simultaneously preventing late blowing. Variable removal of bacteria of this kind leads to even hole formation in the cheese. Shelf life can be extended and maturing time standardized throughout the seasons. The new gentle process for removing bacteria means that the cheese retains the majority of its natural germ flora and thus its typical flavour. Compensating measures, some of them very expensive, such as increasing the addition of cultures or using protective cultures, can be dispensed with.

3.6 Comparison of microfiltration and separator

The following table compares the different types of bacteria removal from milk and rates them from the point of view of quality. This is a qualitative comparison.

<table>
<thead>
<tr>
<th></th>
<th>Microfiltration</th>
<th>Bacteria-removing separator</th>
<th>Double bacteria-removing with separators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Investment costs</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Process integration costs</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lifetime</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Operating costs</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Service costs</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>10 points</td>
<td>14 points</td>
<td>12 points</td>
</tr>
</tbody>
</table>

**Fig. 26** Comparison of microfiltration and separator (+ means beneficial)

3.7 Special applications

The next section goes into more detail about the many applications for bacteria-removing separators which have been tested in the field – specifically for whey. When selecting bacteria-removing separators, assessment of the whey type is the priority. The following remarks highlight a number of options by way of example.

Figure 27 reflects the effect of initial germ content on bacterial clarification effect. Related to total bacteria count, it can be seen that much higher initial values result in a lower bacterial clarification effect. Where species of bacteria were examined individually (spores, Enterobacteriaceae, heat-resistant strains), this is not found. The tests conducted resulted in the following initial values:

- Spores 3 to 30 x 10^3 per ml
- Enterobacteriaceae 0.3 to 10 x 10^6 per ml
- Heat-resistant strains 10 to 15 x 10^6 per ml

Significant deviations in bacterial clarification effect were not found across the full range.

3.7.1 Bacteria removal from whey concentrate

Figure 28 shows the test results for bacteria removal from whey concentrate.

It should be noted that the results were most favourable when output was reduced to approx. 50 percent of the nominal output of the bacteria-removing separators. There was likewise a positive effect when the dry mass of the whey concentrate was not above 25 percent DM. Another effect can be observed in addition to the improvement in quality brought about by centrifugal removal of bacteria from whey concentrate. The bacteria-removing separators act as high-performance clarifiers. In addition to the bacteria, they separate denatured whey proteins, leading to a considerably extended life in the concentration units downstream.
Fig. 27 Bacterial clarification efficiency as a function of the initial bacterial count

![Graph showing bacterial clarification efficiency as a function of the initial bacterial count.]

Fig. 28 Bacteria removal from whey concentrate

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Temperature</th>
<th>Capacity</th>
<th>OM</th>
<th>Cont. concentrate</th>
<th>Discont. concentrate</th>
<th>TBC</th>
<th>Aerobic spores</th>
<th>Anaerobic spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>15 °C</td>
<td>18,000 l/h</td>
<td>27.5 %</td>
<td>80,000 ml</td>
<td>150 ml</td>
<td>0.15 ml</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td>500 l/h</td>
<td>30,400 ml</td>
<td>62 %</td>
<td>19.5 ml</td>
<td>87 % *</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Feed</td>
<td>15 °C</td>
<td>18,000 l/h</td>
<td>30 %</td>
<td>400,000 ml</td>
<td>200 ml</td>
<td>0.04 ml</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td>500 l/h</td>
<td>16,000 ml</td>
<td>58 %</td>
<td>24 ml</td>
<td>88 % *</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Feed</td>
<td>12 °C</td>
<td>15,000 l/h</td>
<td>22 %</td>
<td>220,000 ml</td>
<td>110 ml</td>
<td>0.1 ml</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td>500 l/h</td>
<td>55,000 ml</td>
<td>75 %</td>
<td>9.68 ml</td>
<td>91.2 % *</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Feed</td>
<td>12 °C</td>
<td>15,000 l/h</td>
<td>20.5 %</td>
<td>310,000 ml</td>
<td>190 ml</td>
<td>0.08 ml</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td>400 l/h</td>
<td>86,800 ml</td>
<td>72 %</td>
<td>18.43 ml</td>
<td>90.3 % *</td>
<td>n.m.</td>
<td>≥ 99 % *</td>
</tr>
</tbody>
</table>

* Efficiency
3.8 Bacteria removal – machinery

Bacteria-removing separators are now exclusively equipped with self-cleaning bowls. Customer requirements to reduce the ejection quantities of separators have led to further optimization in this regard in recent years. The GEA Westfalia Separator proplus system features a modified disc stack and solids holding space. By this means, significantly longer production intervals were able to be achieved between the partial ejections, resulting in a reduction of the discharged volume.

3.8.1 Method of operation: bacteria-removing separators

Bacteria-removing separators now also have a GEA Westfalia Separator hydrosoft feed system. The milk for bacteria removal flows through central feed tube (1) into feed chamber (2) which rotates at bowl speed. The feed to disc stack (4) is effected by bores in base of the distributor (3). The bacteria are separated off by centrifugal force. Their higher specific weight causes them to slide outwards into concentrate chamber (5). Bacteria are separated either directly out into the concentrate chamber or on the liquid route inwards as soon as the bacteria reach the underside disc surface of the disc above.

At this point, the speed of the liquid flow in the disc interspace is virtually zero, so that the bacteria are no longer entrained by the product flow. The separated bacteria slide outwards along the bottom surface of the disc under centrifugal force towards the end of the disc and leave the separation chamber. The milk with bacteria removed flows towards the centre of the bowl and is pumped to the discharge point by centripetal pump (6). The bacteria concentrate continuously drawn off flows over separating disc (7) into the top centripetal pump chamber. Concentrate centripetal pump (8) pumps the entrained liquid to the discharge point under pressure and without foam.

In addition to continuous discharge of the concentrate by the centripetal pump, partial ejections also take place. At set intervals, sliding piston (9) is moved hydraulically and part of the contents of the solids chamber are ejected through the discharge ports (10) in the bowl bottom.

Fig. 29 Bowl cross-section of a bacteria-removing separator

---

1. Feed tube
2. Feed chamber
3. Base of distributor
4. Disc stack
5. Concentrate chamber
6. Centripetal pump for milk
7. Separating disc
8. Concentrate centripetal pump
9. Sliding piston
10. Discharge ports

- **Milk**
- **Bacterially clarified milk**
- **Concentrate**
- **Solids**
3.8.2 Method of operation: bacteria-removing separators with GEA Westfalia Separator proplus system

Fig. 30 shows a diagram of the bowl of a bacteria-removing separator with the proplus system. Precisely matched design modifications of the disc stack and separating disc create a supplementary separation zone (4) in this model between solids chamber (3) – this is the space outside separation disc inlet (1) – and disc stack (2). In this area, the bacteria are separated from protein by a specific flow. The protein arrives in the disc stack from outside with the milk which has already had some bacteria removed. The germs remaining in the milk are then separated off in the disc interspace in analogy to bacteria-removing separators without the proplus system.
Example calculation for the commercial benefit of the GEA Westfalia Separator proplus system:

- Annual operating time: 4000 hours
- Protein content of skim milk: 3.36 percent
- Price per litre of skim milk: 0.30 EUR

The following table shows the potential savings from the proplus system with regard to reducing protein losses and the profit which can be achieved as a result.

<table>
<thead>
<tr>
<th>Machine type</th>
<th>CSE 230-01-777</th>
<th>GEA Westfalia Separator proplus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial ejection interval</td>
<td>20 min.</td>
<td>60 min.</td>
</tr>
<tr>
<td>Ejection quantity</td>
<td>8 kg</td>
<td>8 kg</td>
</tr>
<tr>
<td>Protein in solids</td>
<td>9 %</td>
<td>12 %</td>
</tr>
<tr>
<td>Number of annual ejections</td>
<td>12,000 off</td>
<td>4000 off</td>
</tr>
<tr>
<td>Annual protein loss</td>
<td>8640 kg</td>
<td>3840 kg</td>
</tr>
<tr>
<td>Additional annual profit</td>
<td>4800 kg</td>
<td></td>
</tr>
<tr>
<td>Corresponds to a skim milk quantity of</td>
<td>142,857 kg</td>
<td></td>
</tr>
<tr>
<td>Corresponds to an annual profit of</td>
<td></td>
<td>42,857 EUR</td>
</tr>
<tr>
<td>(just as a result of reducing protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>losses)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.9 Machine types
The table shown below lists the current machine types and outputs of bacteria-removing separators for milk. If bacteria are removed from products other than milk, enquire about the corresponding output.

4.0 Sampling
Microbiological tests in milk processing lines can have a variety of objectives: quality controls, seeking possible product contamination or providing evidence of the efficiency of bacteria-removing separators are a few examples. There follow some hints which may be helpful in generating meaningful results.

4.0.1 Prerequisites in the milk processing line
Instruments and control devices in the milk processing line must always work perfectly. Chemical cleaning of the complete line must be guaranteed and validated by visual inspection. All screwed connections must be tight, and no air may be trapped in the product. Flow quantities, pressures and temperatures must be maintained at a constant level during production/testing. The bacteria-removing separator must be adjusted perfectly to suit product quality and feed quantity. This includes the discharge pressure of the milk with bacteria removed, the quantity of concentrate returned and the size and frequency of partial ejections.

<table>
<thead>
<tr>
<th>Machine type</th>
<th>Nominal capacity</th>
<th>GEA Westfalia Separator proplus system</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEA Westfalia Separator ecoClear</td>
<td>7500 l/h</td>
<td>Available</td>
</tr>
<tr>
<td>CSE 140-01-177</td>
<td>15,000 l/h</td>
<td>Available</td>
</tr>
<tr>
<td>CSE 230-01-777</td>
<td>30,000 l/h</td>
<td>Available</td>
</tr>
<tr>
<td>CSE 400-01-777/CSI 400</td>
<td>40,000 l/h</td>
<td>Available</td>
</tr>
<tr>
<td>CSE 500-01-777/CSI 500</td>
<td>50,000 l/h</td>
<td>Available</td>
</tr>
</tbody>
</table>
4.0.2 Arrangement of sampling points
The arrangement of the sampling points depends on the desired test, the arrangement of the line and thus on process management. In principle, the samplers need to be easily accessible. Furthermore, the arrangement needs to be selected so that a representative sample can be taken. If the assessment is only of the bacteria-removing separator, the samplers need to be fitted directly in the feed and discharge of the machine. Samplers may not be fitted in dead spaces or in lines with inadequate flow speeds (< 1 m/s).

4.0.3 Sampling equipment required
Only sterile systems may be used for sampling. Good experience has been acquired using products from JANZ-LABORTECHNIK (Fig. 31), for example. Samplers need to be able to be sterilized with a blowtorch, for example. Sterile containers are required to store the product sample. Sterile instruments – a lance or a syringe and cannula – are required to take the sample.

4.0.4 Performing sampling
A record must be kept in parallel with sampling. This records date, time and name of sample together with all the process parameters and settings. The quantity of product to be taken is based on the desired test methods and should be adequate for this purpose. The instruments required for sampling must remain sterile until immediately before use. The same applies to the sample containers, which need to be labelled clearly with a pen or prepared labels in a manner which can withstand water and wiping. The sampler should be sterilized immediately before the sample is taken. Sampling should be performed rapidly to avoid contamination of the product sample with ambient air as far as possible. The instruments used may not be used again without being cleaned and sterilized again. Fig. 32 shows examples of sterile sampling.

Fig. 31 Samplers and equipment for sterile sampling
Fig. 32 Example of sterile sampling
4.0.5 Treating the samples
Immediately after the sample containers have been sealed to exclude air, the sample must be cooled to a temperature < 3 °C using iced water or dry ice. This temperature may not be exceeded until the samples are examined after no more than 24 hours.

<table>
<thead>
<tr>
<th>Evidence of</th>
<th>Used in (product)</th>
<th>Damage caused</th>
<th>Name of method</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria count</td>
<td>Fresh milk, factory milk, whey</td>
<td>General quality defects</td>
<td>Koch’s plate method</td>
<td>IDF standard 100B: 1991, colony count MB* Vol. VI, 6.3.1</td>
</tr>
<tr>
<td>Aerobic spores of the Bacillus genus, e.g. Bacillus cereus</td>
<td>Fresh milk, UHT milk</td>
<td>Sweet clotting, slime formation, gas formation, swelling</td>
<td>Plate method using GCA agar</td>
<td>MB Vol. VI, 7.17.2</td>
</tr>
<tr>
<td>Anaerobic spores e.g. Clostridium tyrobutyricum, C. butyricum</td>
<td>Cheese milk</td>
<td>Late blowing in cheese, butyric acid formation</td>
<td>Nizo method, setting up dilution series, evaluation as per MPN method, Weinzirl sample</td>
<td>MB Vol. VI 7.18.2 7.18.3 7.18.4</td>
</tr>
</tbody>
</table>

Fig. 33 Typical test methods

4.1 Test methods

4.1.1 Overview
Fig. 33 shows various microorganisms with possible damage caused and test methods typically used. The standards and regulations quoted in the corresponding test methods must also be followed.

4.1.2 Tests for anaerobic spores
The method of determining anaerobic spores in milk is explained in more detail in Fig. 34 (Page 38) by way of an example.

• Preparing the nutrient medium
The nutrient medium is composed of 1000 ml sterile milk and 100 ml glucose/lactic acid mixture. The milk is prepared from skim milk powder (100 g powder to 900 ml H₂O) and sterilized at 115 °C for 15 minutes. The glucose/lactic acid mixture consists of 5 g glucose and 20 ml lactic acid topped up to 100 ml with distilled water. The nutrient medium should have a pH of 5.4 to 5.5. A low pH value favours vigorous growth of the anaerobic spores on one hand and on the other, it inhibits the proliferation of other microorganisms.
• Preparing the test tubes
The test tubes are provided with paraffin rods and sealed with a cotton wool plug or aluminium foil. They are sterilized at a temperature of 121 °C for 15 minutes.

• Selecting the dilution series
The dilution series is based in each case on the number of spores expected. In other words, it is determined by the sampling point (raw milk, milk with bacteria removed). 5 test tubes per dilution series are prepared, e.g. 5 times 10 ml, 5 times 1 ml, 5 times 0.1 ml and 5 times 0.01 ml. It is important that the final sample is negative.

• Preparing the sample
10 ml of nutrient medium are put in the sterile test tubes with their paraffin rods. Depending on dilution, 1 ml, 0.1 ml or 0.01 ml of the sample to be tested are added. Once the test tubes have been filled, they are shaken in a defined manner on a machine. The pH should be checked from time to time. This method also allows 10 ml of sample to be tested. In this case, 10 ml of sample milk and 1 ml of glucose/lactic acid mixture are added to 5 test tubes as nutrient medium.

• Heating the samples (I)
The samples are then heated at 80 °C for a maximum of 5 minutes. The sample must be covered by at least 1.5 cm of water in this process. Undesired germs are killed. The paraffin becomes liquid and seals the test tubes to make them airtight. Cooling takes place in a water bath at 45 °C (II).

• Incubation (III)
Incubation is for 4 days at 37 °C in an incubation cabinet.

• Evaluation (IV)
A sample is considered positive if the paraffin plug has clearly come away from the sample liquid. After counting out, evaluation is performed in accordance with the MPN table.
Fig. 34 Method for testing milk for anaerobic spore-formers
Calculation example

<table>
<thead>
<tr>
<th>Dilution series</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 times 10.00 ml</td>
<td>5 positive</td>
</tr>
<tr>
<td>5 times 1.00 ml</td>
<td>5 positive</td>
</tr>
<tr>
<td>5 times 0.10 ml</td>
<td>1 positive</td>
</tr>
<tr>
<td>5 times 0.01 ml</td>
<td>0 positive</td>
</tr>
</tbody>
</table>

Sample upstream of bacteria-removing separator

- According to the MPN table, this means that the result is 34.8/10 ml

<table>
<thead>
<tr>
<th>Dilution series</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 times 10.00 ml</td>
<td>1 positive</td>
</tr>
<tr>
<td>5 times 1.00 ml</td>
<td>0 positive</td>
</tr>
<tr>
<td>5 times 0.10 ml</td>
<td>0 positive</td>
</tr>
<tr>
<td>5 times 0.01 ml</td>
<td>0 positive</td>
</tr>
</tbody>
</table>

Sample downstream of bacteria-removing separator

- According to the MPN table, this means that the result is 0.199/10 ml

Calculation of bacterial clarification effect

\[
\text{Effect} = \frac{\text{MPN}_Z - \text{MPN}_A}{\text{MPN}_Z} \times 100
\]

\[
\text{Effect} = \frac{34.8 - 0.199}{34.8} \times 100
\]

\[
\text{Effect} = 99.4\%
\]

4.1.3 Tests for aerobic spores

The milk samples are pasteurized in a spin glass at over 80 °C for at least 10 minutes. The sample is then centrifuged in a test tube centrifuge (15 minutes at 1000 g). Once the sediment has been rediluted to 1:10 of its original volume, the bacterial content can be determined using DIFCO NUTRIENT AGAR. It is extremely important to reach the incubation temperature of 37 °C as quickly as possible to facilitate a precise evaluation.

4.1.4 Tests for lactobacilli

The number of lactobacilli is determined using TGV 5.4 AGAR. Incubation time is 5 to 7 days at 30 °C.
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