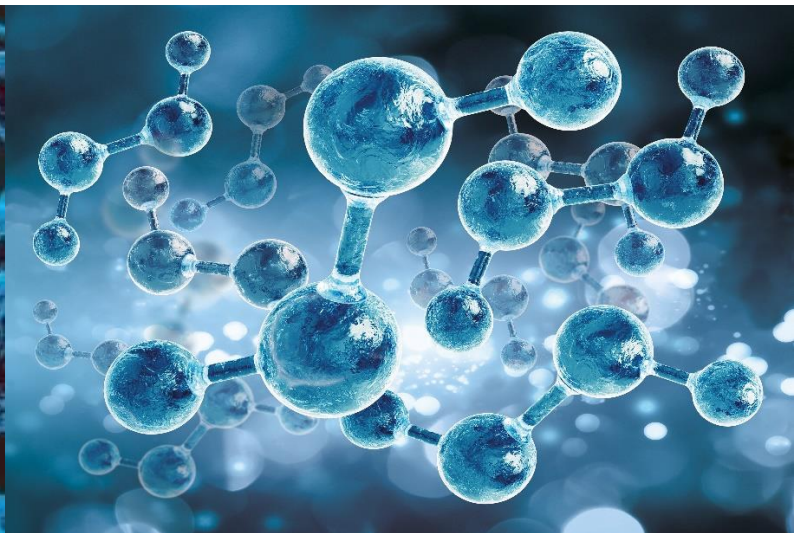


Probiotic and Synbiotic cleaning

***Dr. Robin Temmerman
CEO Chrisal***



Chrisal

Introduction

We are increasingly aware of the fact that a many hygiene problems are due to the wrong microbiology, in which bacteria, viruses or fungi are persistently troubling us. We also know very well the problem of resistance among these microorganisms, as a result of the excessive and inappropriate use of antibiotics and biocides (antiseptics).

Chrisal has been a pioneer in the development of innovative cleaning products based on efficient and sustainable technology for more than 30 years. In addition to a perfect cleaning performance, probiotics and prebiotics are used to create a good microflora that prevents undesirable effects.

The focus is on different domains:

Cleaning Wrong microbiology on surfaces causes increased pollution, odor problems and an increased risk of infectious organisms.

Water Water is the source of all life and always contains a lot of microbiology. Here too, the wrong microorganisms cause major problems such as biofilm, odor and germs.

Air Although air itself does not contain many microorganisms, modern air conditioning and ventilation systems cause major problems with allergens, fungi, fine dust and other contaminants.

The purpose of this document is to provide you with the basic information about the mode of action of our probiotic and synbiotic cleaning products, in which all applications in the above-mentioned domains are explained.

More detailed information is always available at Chrisal or in the specific product information.

In any case, we hope that we can develop sustainable and safe products for you for many years to come and that we can convince you of this wonderful technology.

With best regards,

Dr. Robin Temmerman
CEO

1. Microbiology

Microbiology is the science of microorganisms. A microbiologist is someone who studies microorganisms and searches for solutions based on that knowledge that will improve the lives of humans, animals and the environment.

1.1 What are microorganisms?

A **micro-organism or microbe** is an organism that is too small to see with the naked eye. Only if they are present in large numbers they can become visible. The most important examples of microorganisms are viruses, bacteria, fungi, yeasts and algae. Bacteria are the most common and are approximately 1 micron in size, that is one thousandth of a millimeter (1000 bacteria in a row is no longer than 1 millimeter)!

Microorganisms can be found everywhere in nature. They are found in large numbers on the skin, in the digestive tract, in the soil, in water and in the air.

The majority of microorganisms are benign, useful or even necessary for humans, animals and the environment.

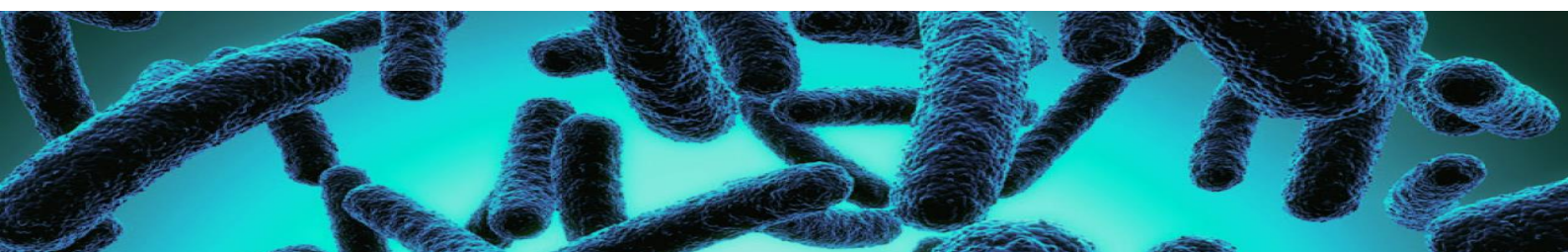
Some examples where microorganisms are very useful:

- Digestion: our food cannot be digested without the many billions of bacteria in our gut
- Composting: dead material from nature (eg leaves, grass, dead animals) is broken down by micro-organisms into the smallest nutrients that are then reused in nature to form new plants or animals.
- Food production: many food products can only be made with the help of micro-organisms such as yeast for bread and wine, or bacteria for yogurt and cheese.

Unfortunately, there are also a number of microorganisms that are harmful to humans, animals or the environment; we call them pathogens. Although they are the minority, they give the microorganisms a very bad reputation.

Some examples where microorganisms are harmful:

- Disease: different microorganisms can cause diseases such as colds, pneumonia, flu, wound inflammation, tetanus, etc. Plants can also be made sick by microorganisms, so that they no longer bear fruit or even die.
- Food spoilage: bacteria in particular can cause spoiled or contaminated food, resulting in intestinal infections and diarrhea after eating this food. Salmonella, E. coli, Listeria and Clostridium are the most important ones.



1.2 The microbial community

Despite their small size microorganisms are very smart. They know how to work together perfectly in order to survive as well as possible. The first traces of microorganisms on earth are already 3 billion years old, so they have much more experience than humans.

Wherever they occur (soil, air, water, animal, plant), microorganisms organize themselves into a community: **the microbial community or microflora**. Such communities can be very diverse and also very complex. Every kind of micro-organism has its task and contributes to the community. Together they only have one goal: to survive as long as possible together.



A more recent term is **microbiome**. This is the total of microorganisms that are located somewhere; whether they work together as a community or not. The best known microbiome is that of humans and includes, for example, the microorganisms present in our mouth, digestive system and on our skin. Even though these microorganisms do not all work together, they do together determine the "state (or health)" of the place where they are (humans, animals, indoor environment, soil, water, etc.). A stable, healthy microbiome is therefore of great importance for our health and that of the entire environment.

When a microbial community is on a solid surface and forms a "layer" there, it is called a **biofilm**. A well-known example of biofilm is the black tile joints in a shower. A biofilm consists of many different types of micro-organisms and all sorts of substances that they produce. These substances can serve as food or as a protective layer against external influences. So the micro-organisms build a house to protect themselves and live together. Unfortunately, biofilm often has adverse consequences for humans. It causes visual pollution, is a source of odor nuisance and is also a hiding place for many germs or pathogens.



What does a microbial community need to survive?

Nutrition: Like any living creature, a microorganism cannot survive without food. Important dietary sources are sugars, fats and proteins. These can be found in many ways in organic pollution (food waste, waste, dust ...) or in sometimes very specific cases such as urine and sweat.

Moisture: Even without "drinking" no (micro) organism can survive. Microorganisms cannot really drink, they absorb moisture from the environment. A bit like a sponge would absorb water. If insufficient moisture is present, their activity will decrease quickly.

Safety: To be able to survive, the environment must also be favorable. By means of the biofilm, micro-organisms protect themselves somewhat against fluctuations in the environment, such as temperature, acidity and humidity.

1.3 The microbial dynamics

The microbial community, microflora, biofilm or microbiome is a living entity; this means that it changes continuously according to the circumstances (moisture, food, temperature ...). Despite these changes, the goal of the microbial community is to stay alive as long as possible. The microorganisms do this by adapting to the circumstances.

Since the microorganisms have an average lifespan of a few days, there will therefore be a continuous dying and growing of microorganisms, the actual composition of the community being determined by the type of food and the environmental parameters.

Unfortunately, the composition of the microflora is not always beneficial for humans, for example when it contains too many members that cause odor nuisance, biofilm pollution or disease. In the last century the knowledge of microbiology was still very limited and people only looked at individual micro-organisms that caused problems. The importance of a stable, healthy microflora was not yet known.

The result was that methods were sought to kill germs or other unwanted microorganisms, without taking into account the good microorganisms. This was the birth of the **hygiene concept**, in which people wanted to live as "clean" as possible. This gave rise to the development of antibiotics and disinfectants, which had a very broad effect with a major impact on the useful microorganisms.



In recent decades, thanks to new techniques, science has gained insight into the importance of the microbial community, rather than the individual micro-organisms. It has become clear that the hygiene concept needs to be urgently reviewed, with the aim of achieving a healthy, balanced microbiome; instead of killing off all microbiology.

In the following chapter we show you the influence of chemical cleaning and disinfection on the microbial balance. Afterwards it is explained what the sustainable alternative of Chrisal entails.

2. Chemical cleaning and disinfection

Because the discovery of microorganisms was mainly linked to diseases, people had the idea that all microorganisms are dangerous. In addition to the search for means to combat diseases (antibiotics), more and more attention was paid to the hygiene of ourselves and our environment. To this end, products have been developed for cleaning and disinfecting.

2.1 Cleaning and disinfection

There is an important difference between cleansing and disinfection, both of surfaces and of our body.

Cleaning: Removing dirt from a surface (a material or our skin). This is done with soaps (or detergents).

Decontamination: Make a surface free of microorganisms by killing them. This is done with biocides (or disinfectants).

Both soaps and biocides are completely chemical in composition, the biocides containing an active substance that is killing micro-organisms. Nowadays, soaps and biocides are sometimes combined, whereby one product should do both cleaning and killing (eg Dettol).

The ultimate goal is therefore to ensure that microorganisms and their food source (= dirt) are no longer present in our environment through cleaning and disinfection.

2.2 The resistance issue

The use of the detergents and disinfectants initially proved to work well and it was fairly easy to make a surface free of dirt and microorganisms. However, due to their long time on earth, microorganisms have learned to adapt rapidly to changing circumstances.

Very quickly (a few decades) after the introduction of disinfectants, the microorganisms found ways to circumvent this threat, which we now call **resistance**. This means that the microorganisms are increasingly able to survive an attack of disinfectants. The efficiency of disinfectants is therefore becoming less and less.

There is also an increasing problem with regard to cleaning agents. One of the mechanisms that the microorganisms use to defend themselves against these chemical attacks is the formation of increasingly tenacious biofilms. As a result, the cleaning agents (soaps, detergents) can no longer efficiently remove dirt from surfaces because it is increasingly stuck in biofilms that are almost impenetrable to soaps.

2.3 The decontamination paradox

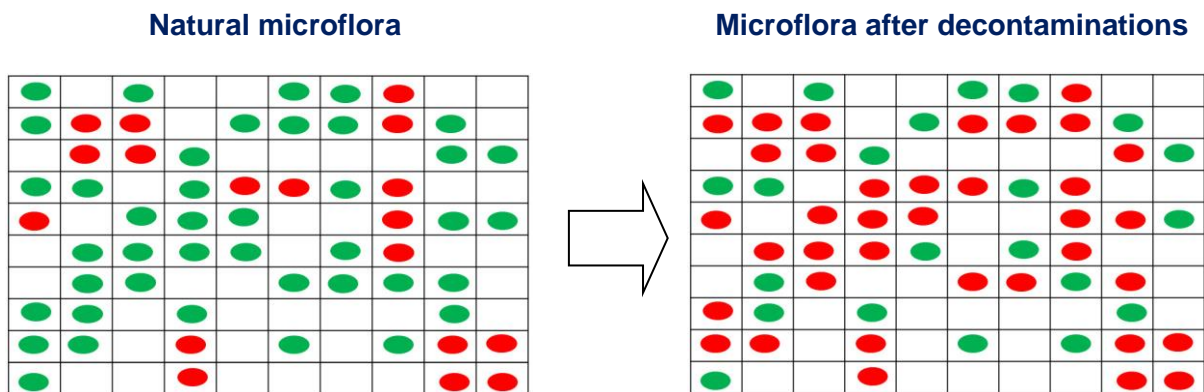
However, there is a greater danger behind chemical cleaning and disinfection. The influence on microbial dynamics is such that the microbial community or microflora is increasingly directed towards a harmful microflora.

What happens with chemical cleaning and disinfection:

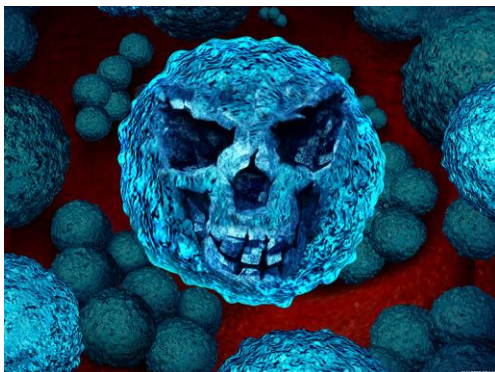
If we take a surface where there is food, moisture and space to keep a microbial community of up to 100 microorganisms alive; immediately after disinfection, the number of microorganisms will have reduced sharply.

However, due to the resistance, a number of microorganisms will survive this disinfection. A disinfectant has no after-effect, so these survivors will start to regrow after a few minutes. After all, they suddenly have a lot of place (the vacant places of the killed microorganisms), food (the killed microorganisms themselves serve as a source of food) and moisture (brought along with the disinfectants).

Since resistant harmful germs survive a disinfection attack best, and therefore grow faster, each disinfection will result in a microbial community that contains more and more resistant harmful germs.



Both surfaces still have the same number of microorganisms, but the proportion of harmful germs is much higher due to resistance problems when disinfectants are used.



The more humans use disinfectants, the more resistant and harmful germs are found.

This is the decontamination paradox.

3. Probiotic and synbiotic cleaning

Chrisal has been aware of the problem of resistance for many years and after years of research has devised a solution for sustainable and efficient cleaning. This solution uses nature and is based on good bacteria (**probiotics**), optionally supplemented with good sugars (**prebiotics**). The combination of probiotics and prebiotics in one product is called **synbiotics**. The probiotic and / or synbiotic cleaners form a healthy microflora during and after cleaning for **optimum sustainable hygiene**.

3.1 How does it work?

As mentioned earlier, the hygiene concept is no longer the radical control of all microorganisms, but the maintenance of a **balanced and healthy microbiome**.

Chrisal's probiotic and synbiotic technology ensures optimum hygiene by (1) keeping a surface clean via a cleaning effect and (2) supporting good microorganisms.

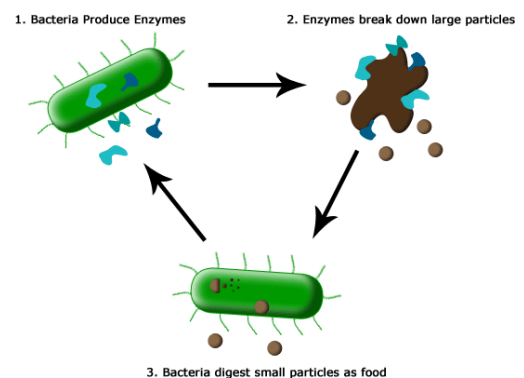
(1) Keeping a surface clean through a cleaning effect:

Cleaning has been at the basis of hygiene for a century. "Cleaning means breaking down dirt, which separates the dirt from the substrate and brings it into a dissolved or colloidal state and makes it easier to remove mechanically and / or physically."

The purer a surface can be kept, the smaller the chance that microorganisms can develop, since they can only feed on pollution. Each type of cleaning and each type of cleaning product will therefore give rise to a lower risk of microbial contamination by reducing the contamination.

Traditional cleaners work via surface-active (chemical) substances that bind to dirt to release it. Later **enzymatic cleaners** were added that add enzymes (biological substances) that act on certain specific contaminants for longer.

Chrisal developed the latest technology in the field of cleaning: **probiotic cleaning**. The effect of the probiotic product is based on the combination of surfactants and probiotics. The major advantage that Chrisal's probiotic products have over traditional chemical or enzymatic cleaners is that the 100% natural probiotic can analyze the surface to see which type of dirt is present. They will then produce highly targeted enzymes on site for days that will break down the contaminated soil and separate it from the substrate and bring it into a dissolved or colloidal state making it easier to remove mechanically and / or physically. This therefore ensures a much wider and longer operation. Probiotic cleaners ensure **maximum cleaning!**



(2) Supporting good microorganisms.

Killing out unwanted microorganisms is no longer the solution. This causes resistance and instability in the microflora. Chrisal's probiotic and synbiotic technology ensures a stable, healthy microbiome through the stimulation of good microorganisms. The high number of probiotics in the products immediately increases the number of good bacteria on the surface (after all, the probiotics are good micro-organisms themselves).



In the case of the synbiotic cleaners, prebiotic sugars are also present, which stimulate the already present good microorganisms through a delayed action. Prebiotics are sugars that can only be digested by good microorganisms. In this way, Chrisal's synbiotic cleansers feed the already present good microorganisms. This promotes the diversity of the microflora in a positive direction.

Probiotic and synbiotic cleaners ensure an efficient, long-term cleaning effect and stimulate good, stable microbiology.

What about the combination of pro / synbiotic cleaning and disinfection?

It may be that a disinfection is nevertheless required, for example due to legal provisions in the healthcare sector, food industry, or as pre-treatment of an air conditioner.

The combination of probiotic and synbiotic cleaning with disinfection is perfectly possible. The disinfectants used will work even more efficiently because the pro and synbiotic cleaners actively remove organic dirt that would otherwise work against disinfectants.

Clean first, then disinfect

For optimum hygiene, ensure that you first clean the material or surface to be cleaned with a probiotic or synbiotic cleaner. This removes all organic pollution. By removing this dirt and grease layer, you can easily reach stubborn bacteria, fungi and germs that are located below with a disinfectant. With a disinfectant you can remove stubborn bacteria, fungi and germs. A cleaning agent does not do this. When the disinfectant has run out, the probiotics of the probiotic or synbiotic cleanser will continue to germinate and provide good microflora and long-term protection.

Even if we strive for a situation where biocides (disinfectants) are used as little as possible; if it is necessary then it is very useful to know that the combination with probiotic and synbiotic cleaning is perfectly possible and makes the disinfection even more efficient. For more specific information about this combination, it is best to contact a Chrisal expert.

3.3 Security

Because probiotic and synbiotic cleansing is a new technology, Chrisal has devoted a great deal of attention from the start of the development to demonstrating the safety of the products and especially the probiotic strains used.

Chrisal probiotics and synbiotics meet the following criteria:

1. All probiotics used belong to ATCC safety class 1 (= highest safety)
2. The pro / prebiotics are 100% natural, not genetically modified (non-GMO)
3. The pro / prebiotics are listed on the list of safe ingredients of the European Food Safety Agency (EFSA) for processing in food
4. Food safe according to the American FDA (GRAS status)
5. Passed the following official OECD safety tests:
 - a. OECD 403 Inhalation toxicity
 - b. OECD 404 Acute skin irritation / corrosion
 - c. OECD 405 Acute eye irritation / corrosion
 - d. OECD 406 Skin sensation
6. The products meet the EU Ecolabel criteria
7. The prebiotics and probiotics meet the criteria for use in cosmetics
8. 10 years of safe use in hospitals and clinical studies



Letters to the Editor

Safety of probiotics used for hospital environmental sanitation

Sir,

There is consensus about the need for efficient control of microbial contamination on hospital surfaces, as these surfaces represent significant pathogen reservoirs that may contribute to transmission of healthcare-associated infections (HAIs). The emergence of multidrug-resistant pathogens in hospitals is a global concern.¹

Control of surface bioburden is routinely addressed by use of conventional chemical-based detergents/disinfectants; however, these are ineffective in preventing recontamination, and may select resistant strains. Recently, cleaning agents containing probiotics of the genus *Bacillus* have been proposed for hospital sanitation [Probiotic Cleaning Hygiene System (PCHS); Copma srl, Ferrara, Italy]; these have been shown to stably decrease surface pathogens up to 90% more than conventional disinfectants, and to be genetically stable even after years of continuous contact with surface pathogens.^{2,3} The rationale for the use of probiotics as sanitizing agents lies in the idea that a healthy microbiota might protect against colonization by, and expansion of, pathogens in the environment as well as in the human body; this has been called 'bidirectional' hygiene.⁴

The three species contained in the probiotic cleansers (*Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus megaterium*) are considered non-pathogenic for humans.⁵ Nevertheless, a theoretical risk of infection exists, and a few anecdotal cases of infection by *B. subtilis* have been reported in surgical patients.⁶ However, systematic assessment of adverse events in probiotic intervention studies is lacking, whereas it has recently been proposed that the most appropriate way to investigate whether probiotics are safe is to use the 'totality of evidence' rather than single case reports.^{7,8} Active surveillance for cases of probiotic-associated infection in all probiotic-based trials has been advocated.⁹ Thus, we have analysed whether the *Bacillus* spp. included in cleaning products may themselves be a source of HAIs. We investigated whether any infections with *Bacillus* spp. occurred in seven healthcare institutions in the province of Ferrara (Italy) that used the PCHS throughout.

In addition to routine culture of all 32,139 clinical samples from around 90,000 patients and 800,000 hospitalizations

days, a quota of samples was also analysed by a *Bacillus*-specific real-time quantitative polymerase chain reaction, as previously described.² The numbers of analysed samples from each institution, as well as the period of environmental sanitation by PCHS, are shown in Table 1. Both culture-based and molecular testing showed complete absence of PCHS-derived bacilli in any clinical sample, for the entire period of the survey. This suggests that probiotic *Bacillus* spp. do not cause infections, even in the subjects at high risk of opportunistic infections.

We think that this surveillance model represents an essential part of the infection control policy associated with the use of probiotics, as it provides ongoing assurance of safety. Accordingly, we are now undertaking a multi-centre study to evaluate a larger number of healthcare institutions for a prolonged period.

Table 1
Analyses performed in the years 2011–2015 in the healthcare structures (HS) continuously using the *Bacillus*-based Probiotic Cleaning Hygiene System (PCHS)

Healthcare structures	Analyses per year (with PCHS sanitation system)					Total analyses (per HS)
	2011	2012	2013	2014	2015	
HS-1	429	—	—	—	—	429
HS-2	103	704	701	613	705	2886
HS-3	—	—	6346	7290	7593	21,229
HS-4	—	76	1025	969	1154	3224
HS-5	—	72	631	713	750	2166
HS-6	—	240	403	498	554	1695
HS-7	—	—	—	—	510	510 ^a
Total^b	532	1092	9106	10,083	11,326	32,139

HS-1, Old S. Anna Hospital (Ferrara), PCHS application March 16th to August 28th, 2011; HS-2, S. Giorgio Hospital (Ferrara), PCHS application since November 1st, 2011; HS-3, New S. Anna Hospital (Cona, Ferrara), PCHS application since January 1st, 2013; HS-4, Delta Hospital (Lagossanto, Ferrara), PCHS application since June 1st, 2012; HS-5, Cento Hospital (Cento, Ferrara), PCHS application since July 1st, 2012; HS-6, Argenta Hospital (Argenta, Ferrara), PCHS application since July 1st, 2012; HS-7, Quisisana Hospital (Ferrara), PCHS application since January 1st, 2015.

^a A quota of these samples was simultaneously analysed also by molecular assays (qPCR).

^b A unique central Microbiology Laboratory (S. Anna University Hospital, Ferrara) performed the analysis by conventional microbiological assays.



3.4 Applications

Pollution and microbiology play a role in many situations and applications. Chrisal has made a wide range of products after more than 15 years of product development. Below is a very brief overview, for more information you can always contact the Chrisal representative.

a) Surface cleaning

Virtually all buildings (private, public or professional) are constantly affected by pollution and odor. The microscopically deep cleaning of the probiotic and synbiotic cleaners ensures a significant reduction of these problems. In addition, building a healthy, stable microflora on the surfaces will ensure a healthier environment.

In addition to cleaning surfaces, Chrisal has also developed a number of products for personal hygiene, including hand soap and shower gel. After all, good skin cleansing with the stimulation of a healthy skin microbiome is very important for good personal protection.

Where: Healthcare sector, agriculture, public sector, schools, offices, shops, sports infrastructure, hospitality, (food) industry, private homes ...

b) Cleaning of water systems

Water is the source of all life and water systems always have to deal with strong build-up of organic pollution due to an unfavorable microflora. This organic pollution causes serious problems such as odor nuisance, discolouration of the water and in particular clogging of pumps, pipes and filters. For technical installations, this can result in a large loss of efficiency or result in high maintenance costs.

The probiotic and synbiotic cleaning products for water systems ensure that organic dirt is digested and released so that it is removed more quickly by the physical movement of the water. The stable microbiology that is formed ensures a lower risk of smelly and cloudy water.

Where: Cooling water, ponds, rinsing water, production water

c) Cleaning of air systems

Although air contains far fewer microorganisms, modern air conditioning and ventilation systems can also cause an accumulation of organic pollution in which, among other, allergens are present. This causes unhealthy indoor air in the buildings concerned that often gives rise to the "sick building syndrome". The probiotic and synbiotic cleaning of air systems digests dirt in the air ducts so that the physical air flows can drain it. This keeps the air ducts clean and the pro / synbiotic cleaning also ensures a healthy microflora on all surfaces in the interior.

Where: Professional and private HVAC systems; car / truck / airplane air conditioning

4. What makes Chrisal products unique?

In order to enjoy the maximum effect and benefits of probiotic and synbiotic cleaning, the products must meet a number of important criteria:

Composition of the product

The selection of the types of probiotics and prebiotics are crucial for the proper functioning of the product. A wide variety of species is available and it is very important to know what effect a certain species has under certain circumstances. Chrisal has been doing research for many years to select the best performing probiotic bacteria and prebiotic sugars for the desired application. For example, a probiotic Lactobacillus, known from the probiotic nutritional supplements, will be practically worthless for environmental applications. The Bacillus species used by Chrisal, originating from soil and water, are ultra-efficient!



Product stability

The number of pre- and probiotics in the product is also of great importance. Often the organic pollution on a surface is very strong. A probiotic cleaning product with only a few probiotics will not work. The Chrisal products contain an absolute minimum of 50 million probiotics per ml. Certain products go up to half a billion per ml!



Probiotics and prebiotics must also survive the entire life of the product. Chrisal is by far the market leader in terms of stability and gives all of its products a shelf life of at least 3 years!



Product quality

To guarantee the proper functioning and safety of every product, a sound quality system is of great importance. Chrisal has the ISO9001 quality certificate since 1999. This guarantees the proper functioning of the entire company.

In addition, Chrisal has 2 laboratories that are equipped with all the required modern technology to be able to carry out thorough quality control. Producing products with probiotics is not easy and guaranteeing purity requires modern equipment and specialized lab technicians.



Product Operation

The operation of all Chrisal products is always externally validated by specialized academic or private institutions. Chrisal's regular research partners include UGent, Eurofins, SGS and BMA Labor

Conclusion

With the probiotic and synbiotic cleaning products, Chrisal has found a true revolution and proven solution in terms of cleaning. The optimal effect in combination with their safety, durability and environmental friendliness make these products the sustainable hygiene that we all need.

In the coming years, this technology will be used in more and more sectors. You can help to build a sustainable future!

Be part of the difference for a better world!

