THE PRINCIPLE OF RESIDUAL BARRIER TECHNOLOGY (RBT247)

Why microorganisms only and not the others?

No RBT 24/7 Effect

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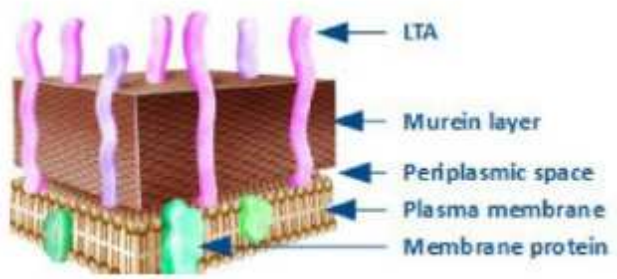
100 % Kill Rate

Animals and plants are multicellular organisms where the single cell is protected by its neighbours. Membrane active antimicrobials applied at micro or nano concentrations might affect a single cell at organism`s periphery but will never affect the whole organism.

Bacteria on the other hand are single cells but can live in greater communities - so called biofilms.

The cell membrane of bacteria is their most important protective shield against the environment.



Lipoteichoic acid (**LTA**) is the toxic tentacle of Gram-negative bacteria.

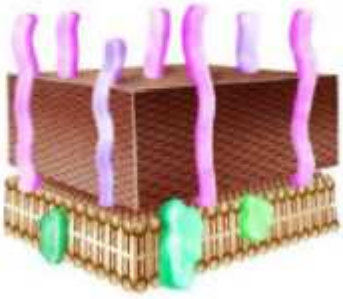
This tentacle is used to defend other cells, to communicate with the environment, etc.

The murein layer consists of multiple single peptidoglycan sheets forming a gel-like structure.

For more details see below.

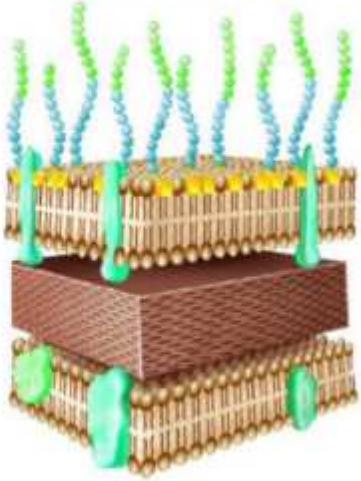
The plasma membrane is formed by two phospholipid layers in which proteins and pores are incorporated.

Monoderm bacteria

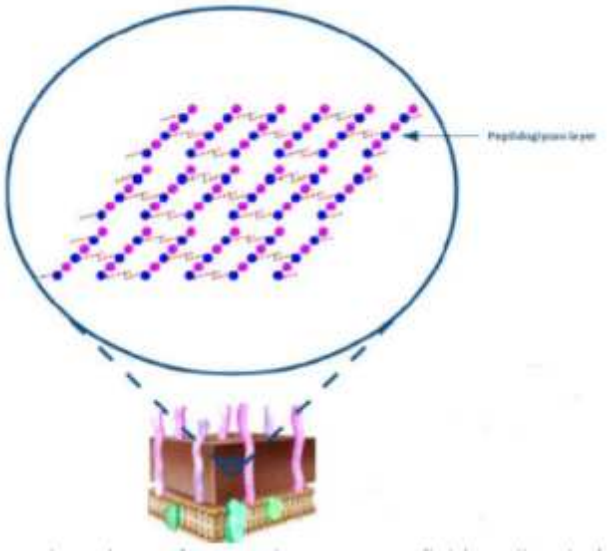


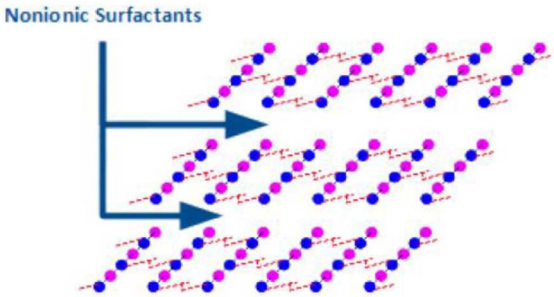
Streptococcus, Enterococcus, Staphylococcus, Listeria, Bacillus, Clostridium, Lactobacillus etc

Diderm bacteria



Escherichia coli, Salmonella, Shigella, Klebsiella, Proteus, Enterobacter etc





Although the murein layer looks like a solid block the consistency is weak and gel like.

The different peptidoglycan layers are not chemically bound to each other.

Intercalation by surfactants - e.g. nonionic surfactants as applied within RBT 24/7 - can easily remove single up to multiple layers.

Monoderm bacteria are the most assailable cells. Their most prominent defence ability is represented by the toxic tentacle lipoteichoic acid (**LTA**).

Lipoteichoic acid (**LTA**) contributes most to the high toxicity of some monoderm bacteria like *Staphylococcus, Listeria* or *Clostridium* while others - e.g. *Lactobacillus* - are beneficial and used in food processing.

Furthermore, the lipoteichoic acid (**LTA**) is zwitterionic and allows the bacteriae to stay in a watery environment.

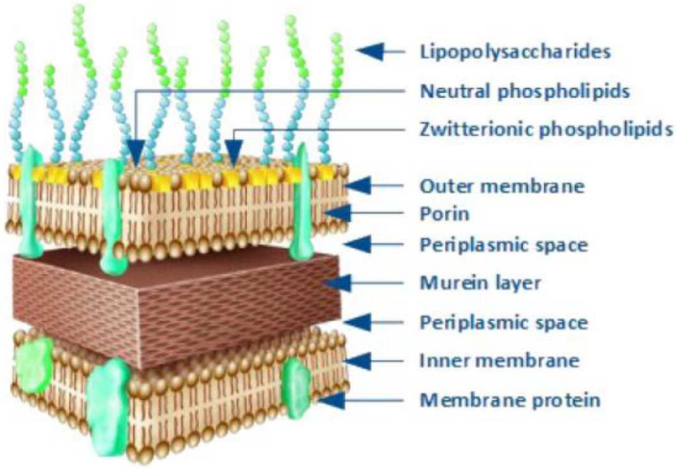
The thick murein layer shows only reduced defence capability as it can easily be peeled off by surface active chemicals. The plasma membrane itself is not a solid barrier.

The phospholipid bi-layer can be penetrated easily by proteins and other biomolecules.

To fight monoderm bacteria two tactical objectives should be followed.

- Peeling off the murein layer
- Removing phospholipids from plasma membrane

When this tactic is applied, monoderm bacteriae are easy to kill.



Diderm bacteria have a sandwich like cell wall where the murein layer is sandwiched by two membranes - the outer membrane comprising special phospholipids and the inner membrane which is in principle common to all cell types - of microbes, plants and animals.

Because of this special structure diderm bacteria are stronger and therefore harder to kill.

The outer membrane of diderm bacteria comprises of the exterior side neutral and zwitterionic phospholipids.

These zwitterionic phospholipids allow the micro-organism to stay in a watery environment.

The interior side of the outer membrane is built up of neutral phospholipids only.

To guarantee the transport of nutrients and other biomolecules the outer membrane is provided with pores - so called porins.

The murein layer of diderm bacteria is less pronounced compared to monoderm bacteria as the protective issue is less significant because of the protective function of the outer membrane.

The inner membrane is directly comparable to that of monoderm bacteria

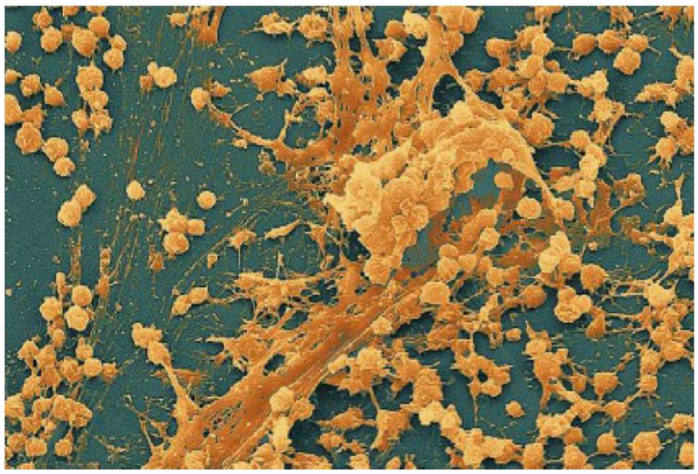
To fight diderm bacteriae three different steps must be applied:

- Opening the outer membrane by removing zwitterionic phospholipids
- Removing residual neutral phospholipids
- Peeling off the murein layer
- Removing phospholipids from the plasma membrane

When this tactic is applied, diderm bacteria are killed.

In real life the situation is more complicated as microorganisms are protecting themselves by building up a biofilm.

Bacteria are the archetype of settlers. Once they have contacted solid ground they will settle down, find a grip and start growing.



When living conditions are at the optimum, one bacterium will produce 1 million of successors within 6 hrs.

But their life is not easy, only the fittest and strongest will survive.

The rest ends up as part of their protection system - the biofilm.

Biofilms are a conglomerate of living cells, bacterial waste, proteins, peptidoglycans and other biomolecules.

Biofilms are the prototype of multicellular organisms where single cells contribute to the living community by fulfilling special tasks.

Biofilms are limited in growth depending on nutrients in their micro environment.

Therefore, single cells will be dispersed into the environment to settle down anywhere else.

The strategy in fighting bacteria is a multitasking procedure starting with the disintegration of biofilms and ending in destroying all single survivors.

To get rid of this sticky lump simple flushing with water will make them only stronger.

The first strategic point of fighting is the detachment of the sticky material from the ground.

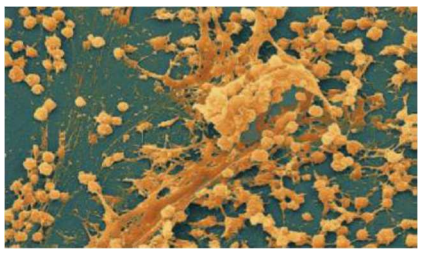
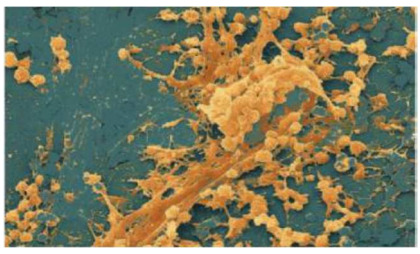
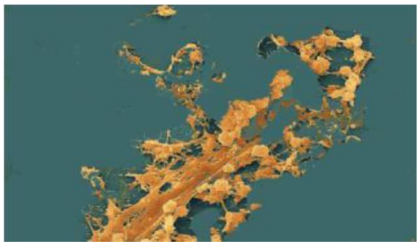
This job is done by the ionic and non-ionic surfactants of RBT 24/7. Once enveloped, the dirt can easily be flushed away with water.

As RBT 34/7 comprises also antimicrobials, killing of micro-organisms is an ongoing job.

The destruction of a mature biofilm is not done by a single application of RBT 24/7.

To get rid of a persistent biofilm a treatment period of around 1 week is required.

Once the green (see below) is reached, daily application of RBT 24/7 will keep the green living.

		
Start	Day 2	Day 4

Day 6

Day 8

From our experiences in the fish and poultry processing industry, we know that the above described fighting strategy is not a sandpit manoeuvre.

A further crucial part of this fighting strategy is the application of surface active biocides via a special nebulizer.

The nebulizing technique is not a special IP.

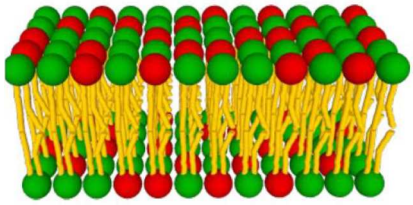
Only the use of RBT 24/7 makes it unique.

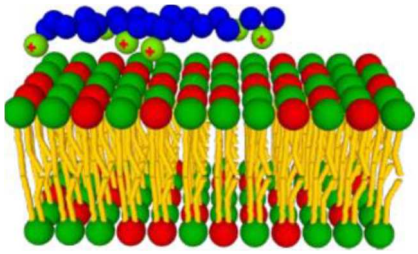
The final step is the killing of the biofilm members. How to affect the protective murein layers is described above.

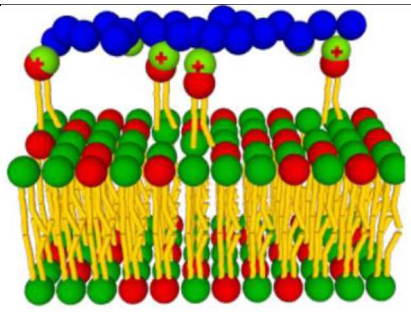
The targets are the different membranes which must be opened to let the organism bleeding out.

The first attack is done by the antimicrobial PHMB-Polyhexamethylene Biguanide. PHMB will selectively pick up zwitterionic phospholipids of the cell membrane.

As these zwitterionic interactions are weak, the picked up phospholipids will be utilized by non-ionic surfactants to form mixed micelles







Although the concentrations of PHMB used are at lowest level, the antimicrobial molecule exceeds the number of bacteria by a factor of about 1 billion (10^{9}) per µl while traditional chemical biocides are in the range of 1 quadrillion (10^{15}) per µl and higher.

To finalise, the nonionic surfactants are adjusted to 1 trillion (10^{12}) per µl which is still fair in respect to the environment - especially as these surfactants are of natural origin

The nonionic surfactants support the biocidal activities of the antimicrobial peptide.

Firstly the nonionic surfactants must have CMCs (Critical Micelle Concentrations/Concentrations where the surfactants starts to form micelle) within the mM range.

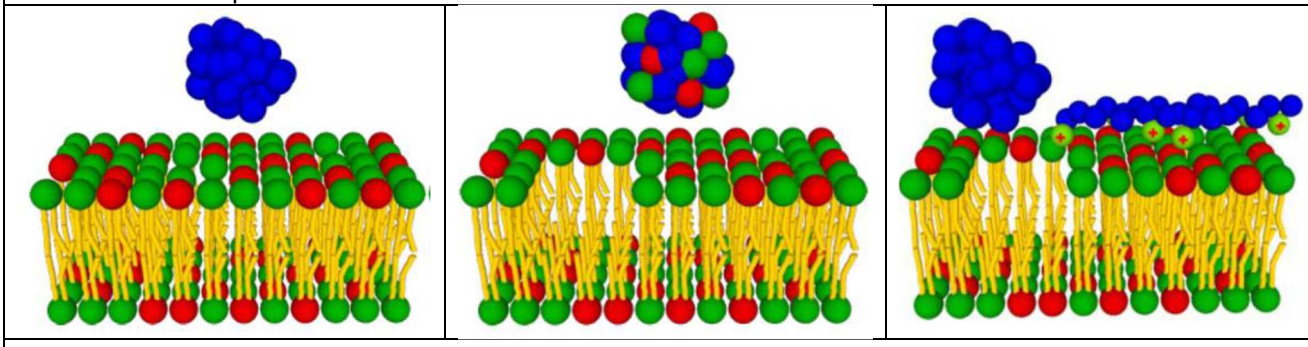
Most of the nonionic surfactants used in cleaning etc. have CMCs in µM range.

Secondly, as 2 nonionic surfactants are applied both should not differ in their CMCs by a factor of 10.

CMCs of 1.5 and 17 are used in RBT247.

With this combination there is a broad transition phase between both nonionic surfactants where less stable mixed micelles are formed.

Thirdly the non-ionic surfactants must form mixed micelles by incorporating phospholipids of the cell membrane. In simple words: Micelles eat bacteria.



A further antimicrobial compound is given by the quaternary ammonioum compounds which are added at a low level.

These antimicrobial quaternary compounds attack the cell membranes via ionic and hydrophobic interactions and their mechanism is comparable to the mechanism described above.

There is a broad experience repertoire regarding application of the biocidal mixture as well as that of the nebulising system.

Most of the field tests have been performed in fish processing industry and most of the experiments were done at University Erlangen and MATIS institute in Reykjavik

BENEFITS OF USING RBT247

- Kills all known Bacteria, Viruses and Fungal Fast
- Eliminates all harmful pathogens to >log 7 at point of application (99.99999%)
- Stops mutant strains from occurring
- Continues to kill harmful pathogens 24/7 using unique residual barrier technology
- Binds to the surface creating an active barrier
- Highly effective even in soiled conditions
- Works when dry
- Gentle to use on humans
- Gentle to use on animals
- Gentle to use on all surfaces
- Alcohol free