FREQUENTLY ASKED QUESTIONS

Do enzymatic products guarantee a more effective cleaning?
Realco/Realzyme validated the effectiveness of its formulas through a reliable measuring method. Quantitative tests on the cleaning and disinfecting effect have been conducted by known organizations like “Institut Meurice”.

The combination of the enzymatic cleaning and the sanitizer enables us to obtain a superior level of cleanliness and disinfection.

Do enzymes work immediately?
Yes. It has been demonstrated, through many references, that the hydrolysis action of enzymes is instantaneous when placed in *good conditions* of pH and temperatures. Depending on the applications, the substrate to degrade, the pH and temperatures conditions, Realco/Realzyme has selected enzymes that will guarantee a maximum effectiveness.

How long are enzymes active?
Depending on the enzyme itself and the formulation, they are active, and will continue to work, for 12-24 hours.

What temperatures do enzymes work at?
The *optimal* temperature for the enzymes is between 104-130ºF (40-55ºC).

What is the optimal pH range for enzyme-based detergents?
Depending on the enzyme and formulation, the optimal range is 5.5 to 8.

Do enzymes do not leave any residual deposit on surfaces?
- A study (in collaboration with UCL and INRA) demonstrated the absence of residual enzymes on different types of surfaces after the cleaning procedure. The process consisted of:
  - Soaking strips of stainless steel in an enzymatic solution, then rinsing.
  - Analysis of the composition of the surface elements by spectrometry.

Observation: Cleaned surfaces show very low nitrogen levels = proteins absence = absence of enzymes.
With the absence of residual enzyme, risks of interaction with the flows or products in contact with the surfaces are brushed aside.

**Is it possible to deactivate enzymes; how do you deactivate enzymes?**

Yes, there are a variety of methods. The simplest method is to allow them to dry out. If they are not maintained in aqueous state, they will deactivate. Other methods are:

- Increase temperature to above 170°F (77°C)
- pH level change: A dramatic increase (alkaline environment, above 10 pH) or decrease (acid treatment, 2-4 pH)
- Use of a sanitizer with oxidizing agents (like chlorine-based products). Quat-based products are not ideal for fully deactivating enzymes. Others, such as alcohol-based, are not effective in deactivation.

**Does the Biorem product range remove biofilms?**

In combination with registered sanitizers, biofilms can be completely removed from any surface. The Biorem product cleans and prepares the surfaces for the application of the sanitizer.

**Can enzymes be used in combination with sanitizers and/or acids?**

No, these will deactivate any enzyme activity. They must be used separately, before or after, the application of the enzyme-based product.

**What are the operating conditions?**

- Realco enzymatic detergents designed to clean the open surfaces of equipment are used at 1-3% concentration in hot water (95-122°F / 35-50°C).
- Enzymatic products are applied in exactly the same way as standard products: foam gun, manually-wash, soaking, etc.
- For each special method of application, a risk assessment must be carried out and approved by our main supplier of enzymes.
FREQUENTLY ASKED QUESTIONS

This graph by UCL and INRA indicates that an enzymatic product (Degres-L, 1%) rinsed by the standard procedure leaves a low level of residue, equivalent to a surface washed with potable water.

In contrast, non-formulated enzymes are less easily flushable.

However, if the product is poorly rinsed on a surface that is in contact with food products, this can be detrimental to the food coming into contact with the enzymes. This is also valid for conventional chemicals.

Adding a disinfectant is required after an enzymatic cleaner has been used, for 3 reasons:

1) Create the necessary oxidizing effect to deal with "oxidizable" stains
2) Destroy all "normally present" bacteria as well as the bacteria released by the biofilm matrix
3) Irreversibly inactivate residual potential enzymes

What is the efficiency of enzymatic products compared to conventional chemical formulations; what are the optimal conditions of use for enzymatic detergents knowing that the optimal conditions of enzymes are observed at high temp?

The efficacy of enzymes is equal to higher chemical formulations containing soda or potash used in F&B applications. A two-year European project, in partnership with INRA Lille and UCL (Université Catholique de Louvain), has demonstrated the real value of thorough cleaning of enzymatic cleaners compared to conventional cleaners. Several factors weigh in favor of its results:

- The temperature kinetics of chemical hydrolysis are generally higher than the temperature of enzymatic hydrolysis. In addition, the enzyme is a catalyst, i.e. it remains intact after each hydrolysis reaction and can be reappointed in several
successive reactions while consuming chemical hydrolysis product and neutralizes over time.

- The optimum conditions for the use of enzymes are at a temperature 113°F ±5 (45°C, ±5). Nevertheless, the enzymes are already beginning to be active at 50°F (10°C) (cold water).

The below graph resulting from project NETZYM / UCL-INRA, is a comparison of a conventional alkaline-based cleaner (pH 11.2) and enzymatic cleaning of a soiled surface with a complex of starch soil. After (5) minutes of action, enzymatic cleaner removed (3) times more dirt and continues to act in time while the alkaline cleaner saturates after (15) minutes.
What is the impact on WWTP if enzymes are not degraded?
The enzyme is a protein and is therefore 100% biodegradable. A hydrolase enzyme is active, but it is for a defined time. The three-dimensional structure which gives the specific activity of the enzyme is destabilized by many external factors such as changes in pH, temperature, and water itself (hydration of certain groups and nucleophilic substitution on certain chemical groups). Realco found that activated enzyme lost almost all of its activity after 48 hours in a non-aqueous medium stabilized.

Finally, our laboratory measures the biodegradability of all conventional and enzymatic detergents via the standard OECD 302B. We are therefore able to report that all of enzymatic detergents from Realco are biodegradable and average more than 95% in 28 days (Degrees-L: > 98%, 3% Enzyfoam > 99%).

How can we measure the degradation of enzymes? Also, why does an enzyme degrade organic matter such as food but does not impact living organic matter (such as the skin of employees!)?
Degradation of enzymes is measured by the loss of enzyme activity over time. A solution that contains more enzyme activity is a solution whose enzymes were inactivated irreversibly. From that moment, the enzyme becomes a mere waste that will biodegrade like any other waste. Enzymes used in detergents hydrolyze protein, fat, starch and fiber. A pure enzyme (undiluted detergent) is an irritant and allergen but is not corrosive. It does not attack live tissue as corrosive compounds would though.

Are there any settings issues to be aware of with the use of enzymes (t°, air humidity level, etc)?
A recurring problem parameter is the temperature of equipment and ambient air in general. In fact, low temperatures decrease the rate of enzyme kinetics as well as alkaline products.
FREQUENTLY ASKED QUESTIONS

What is the shelf life of enzyme-based products?
Powder products have a 24-month shelf life for guaranteed activity; liquid products have a 12-month shelf life. Beyond those timeframes, the products are still acceptable for use although the concentration may need to be increased minimally.

After a liquid product has been opened, full activity is only guaranteed for (6) months. Always be sure to close the product lid tightly, on both liquid and powder products, to prevent moisture from entering and starting enzyme activity.

What are the storage conditions for enzyme-based products?
Ideal storage is in the original closed packaging, at temperatures between 40-77ºF (4-25°C). If the product freezes, once it is thawed, it is usable. If the temperature exceeds 170ºF (77ºC), the product will no longer be usable.

Why is there alcohol content in the enzyme formulations?
Alcohols are generally used as elements guaranteeing the stability of enzymes. They also have a degreasing effect in a detergent formulation.
Frequently Asked Questions

Safety

Are enzymes safe?
Novozyme and other major enzyme manufacturers gathered much literature proving the safety related to the use of enzymes in detergent products (toxicity, skin irritation, allergy).

• The use of enzymes in cleaning product is safe for the user.

In order to reassure the user and to offer safety guarantees, Realco/Realzyme provides information based on:

• Scientific literature
  o Enzymatic detergents do not increase respiratory sensitization (asthma)
  o Manipulation of enzymatic product is not dangerous
  o Enzymatic cleaning does not require specific protections

• Independent studies
  o Enzymatic detergents are considered as non-irritant
  o Enzymes products applied with a spray do not increase the risks of allergy by inhalation

Are enzymatic products considered non-irritant?
Skin irritation tests have been carried out with ENZYFOAM: an enzymatic foaming degreasing agent for floors and surfaces, applied with foam units or guns.
These experiments were carried out by a French laboratory that specializes in safety and tolerance testing and in specifications for detergent and cosmetics products: EUROSAFE.

1) Skin toxicity test on explants of fresh skin (PREDISKIN model)
Principle: Contact between Enzymousse 1% and skin samples.
Expected results: If cellular viability is under 50%, the product is an irritant. If cellular viability is over 50%, the product is not an irritant.

This test, supervised by a dermatologist, has confirmed that the product has been tested under dermatological control if the product causes no reaction.
FREQUENTLY ASKED QUESTIONS

2) Principle: (21) healthy volunteers are in contact for 48 hours with patches impregnated with ENZYFOAM at 1%.
Expected results: Scoring system by a dermatologist according to the degree of irritation.

Results: The (21) volunteers scored “0”. ENZYMOSUSSE at 1% is declared a non-irritant. Furthermore, this test supports claim that the product in application has been "tested under dermatological control".

Realco/Realzyme ran tests on Degres-L and Degrazym products as well.

1) Evaluation of the skin irritation’s risk on pieces of human skin: Predskin test

The 2 products are declared “non-irritants”

2) “In vivo” study: Evaluation of the skin irritation risk thanks to a patch test placed on volunteers.

Evaluation after 48 hours: Products are declared “non-irritant” and tested under dermatological control

Are they dangerous to use and/or handle?
• Enzymatic detergents are neutral, non-corrosive and non-volatile so they present no particular risk.
• They are even less dangerous than most professional soda-based cleaning agents, oxidizing agents, acids, solvents or quaternary ammonium compounds.

Is any special protective equipment required?
• Enzymatic detergents are by no means aggressive, but they are detergents nonetheless and are classified as "irritants" due to their complex formulae.
• So, as with any product in this class, the elementary safety rules in the workplace must be observed.
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Is there any risk of allergies?

• In the 1960s, enzymes first appeared in the world of cleaning (detergents) and some cases of allergy were reported.
• To prevent fine enzyme powders from reaching the respiratory mucosa, the industry encapsulated the enzymes with a thicker coating to avoid both the airborne enzymes phenomenon and direct contact between enzymes and the consumer.
• Purification techniques have resulted in enzymes of higher quality that are less of an irritant.
• There is only one official occupational exposure limit. The value of 60 ng/m³ is confirmed by AISE and should be considered either in TWA (8 hours time-weighted average) or STEL (15 minutes short-time exposure limit) according to country. However, the leading suppliers of enzymes require lower mandatory limits, set at 15 ng/m³ in TWA.
• Within the framework of the new REACh legislation on raw materials, enzymes suppliers for self-foaming enzymatic products plan for exposure scenarios. Realco has also been actively consulted by its suppliers to draw up exposure scenarios.

What are the conditions of use, handling, preparation and application and in rinsing enzyme solutions? What type of PPE should I wear and why?

Enzymatic detergents from Realco/Realzyme can be used in open areas from 0.5% to 2% with hot water (95-122°F / 35-50°C). The time of action of enzymatic detergents varies between 5 and 30 minutes depending on application conditions (scrubber, foam gun, etc) and media.

The enzymatic detergents are generally classified R41, handling therefore requires protective equipment hands, eyes and face. The use of the products in the conditions of use recommended by Realco is safe for the user. Skin irritation tests were carried out by the French laboratory specialist in cosmetic safety, Eurosafe. Two specific tests were performed: 1) Tolerance test ex-vivo human skin explants (model "prediskin") and 2) 48 human patch test on a significant sample of volunteers. They allow us to market our range of enzyme products as "non-irritating, dermatologically tested".
Modes of applications of enzyme products are exactly the same as conventional products: scrubber, foam gun, etc. For each specific application mode, a risk assessment has been completed and approved by our main supplier of enzymes. In the specific application of self-foaming products, the levels found are below 15 ng/m$^3$ and ensure the safety of the professional user. However, according to the standards for respiratory protection (EN 140, EN 143 and EN 149), when there is a risk of aerosol generation for a detergent containing hazardous materials, wearing a mask or half-mask with P filter is required. In the case of an enzymatic detergent, the mask is provided with a filter P3 (filtration of solid and liquid aerosols) as well as products containing a substantial proportion of sodium hydroxide and / or potassium hydroxide and / or sodium hypochlorite.
FREQUENTLY ASKED QUESTIONS

APPLICATIONS

Are enzymes inactive after a membrane filtration unit cleaning process?
Realco/Realzyme studied the processes of enzymes inactivation after the membrane filters cleaning:

- It demonstrated that the (4) types of hydrolase's (specially selected accordingly to the substrate to be degraded) used during the enzymatic cleaning, are easily inactivated by a thermal or chemical shock.

The inactivation type will be chosen based on energy costs, type of membranes and the specifics of the installation.

What is the most effective mixture of enzymes?
- Realco enzymatic products intended for the food-processing industry are standard detergent products to which enzyme mixtures are added.
- Generally speaking the detergents routinely contain a mixture of stabilized enzymes that attack proteinic, fatty and starchy stains.
- These mixtures cover wide temperature ranges because several enzymes can be selected within any one group.

What are the limitations of use?
- The most common limitations are the temperature of equipment and of the air in general. Low temperatures slow down the enzymatic kinetics in the same way as alkaline products do.
- Enzymes are incompatible with most biocides on the market – they can’t be used together (apart from certain quaternary ammonium compounds or amines).
- A strong oxidizing agent is required when using enzymes for cleaning. Certain processes are sensitive to their presence and adding an oxidizing agent disinfects and inactivates the residual enzymes.

Does the recommended temperature always have to be 50°C?
- It all depends of the enzymes selected for the different types of applications. The Temperature-Activity ratio for enzymes is described by a curve. The slope varies according to the enzyme.
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- Realco has selected enzyme mixtures that work at 104-122ºF (40-50°C) with differences in activity in the region of 10-20%, given that maximum activity is achieved at 104-122ºF (45-50°C).
- Besides enzymatic activity, one should not forget that temperature also plays an important part in the TACT (temperature, action, concentration, time) rule.

How can one measure enzyme breakdown?
- A solution that contains no more enzymatic activity is one solution in which the enzymes have been irreversibly inactivated. From that point, enzymes become biodegradable waste.
- Enzymes used in detergency hydrolyze proteins, fat, starch and fibers. A pure enzyme (not diluted in a detergent) is non-corrosive matter.
- A protease-based detergent coming in contact with a piece of meat will start to break the meat down hydrolyzing the proteins.

What are the environmental risks?
- There are no enzyme-related environmental risks. On the contrary, the hydrolases used in enzymatic detergents are the same enzymes secreted by bacteria in the water purification process.
- In other words, enzymes used in cleaning products do not retard the water purifying process as a conventional alkaline detergent may.
- Alkaline products can cause malfunctions in the wastewater treatment plant and neutralize the pH. Furthermore, this can momentarily reduce the biodegradability of effluents.