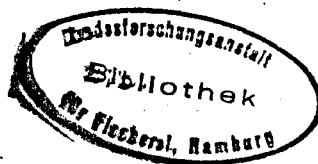


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Toxicity of Proteolytic Enzymes as Additives
to Washing Compounds

by
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During recent years proteolytic enzymes have been utilized to a great extent as additives to washing compounds. How significant these enzymes are is best expressed by the fact that today more washing compounds with enzyme addition exist than without. Proteolytic enzymes as additives are supposed to digest the proteins in textiles, e. g. mainly dirt and stains caused by blood, milk, chocolate, and other substances containing protein. According to Adam, Thirnagand and Schmitt (1970) 0,1 to 1,0 % of the washing compounds consist of enzymes. They are bound to a carrier, usually phosphates.

With household and industry sewages detergents enter the waters. It was, therefore, of interest to find out how the enzymes affect fish and other organisms of fresh water and sea-water.

For our investigations we used the proteolytic enzyme MAXATASE P produced by the Koninklijke Nederlandsche Gist-en Spiritusfabriek at Delft/Netherlands. According to information received from this firm the proteolytic enzyme in question contains 300 000 Delft Units (DU). Apart from the proteolytic enzyme it also contains small quantities of an enzyme which liquefies starch. Hereby the cleaning effect is improved. It has an optimum activity at pH6 to pH8 and normal water temperature (10-25°C). A decrease of the activity does only occur at a temperature of over 50° C.

For our investigations we used samples of the following fresh water and brackish water animals:

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Tubifex (worms)
Lebistes reticulatus (aquarium fish)
Salmo gairdneri (rainbow trout)
Rivulus cylindraceus (aquarium fish)
Anguilla anguilla (eels)
Gammarus salinus (brackish water crab)

The investigation results are given in the summary mentioned below. The lethal dose (LD₅₀) over a period of 24 hours is as follows:

Rivulus cylindraceus	1 mg/l
Trouts	5 - 15 mg/l
Lebistes	25 mg/l
Tubifex	50 mg/l

These values concern fresh water animals. Our investigations have shown that fish eggs and fish fry are especially easily affected. In these cases already 1-5 mg/l would prove lethal. During the hatching of the eggs of Rivulus and rainbow trout it was observed that the eggs are very quickly attacked by the fungus Saprolegnia. It is thought that the proteolytic enzyme attacks the surface of the eggs. The results of the investigations on eel (eel fry of 6-8 cm), which were carried out in fresh water, brackish water (13,7 ‰ salinity), and sea-water (26,9 ‰ salinity), are very interesting. LD₅₀ was reached in sea-water at 7,5 mg/l, in brackish water at 20,0 mg/l, and in fresh water at 30,0 mg/l. It is felt that the activity of proteolytic enzymes may possibly be influenced by the pH-value of the solutions. The maximum activity is obtained in the alkaline field, whereas a reduction in activity takes place towards the neutral point (pH 7). It is, therefore, believed that the activity of the enzyme is less in fresh water (pH 6,8) than in sea-water (pH 8). The brackish water gammarids tested could endure higher concentrations (200 mg/l). This is an observation which is known from other toxicity investigations.

The question now arises as to the amount of proteolytic enzymes contained in natural waters. By the heating of the washing solution during the boiling process up to over 50°C the enzymes are very largely destroyed so that only small quantities get into the sewages. This remainder is destroyed in the purification plants. It is, therefore, not to be expected that under normal conditions proteolytic enzymes in washing compounds will cause toxicity.