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Measuring the action of washing product proteases

An easy way to monitor protease activity

Proteases in 'biological' washing powders

At the start of the 20th century, German chemist Otto Röhm was trying to solve an unpleasant problem. He was trying to develop an improved method of 'bating', the process by which animal hides are softened before tanning to make leather. The motivation behind this work was that the substance then used for bating was highly offensive — dog faeces.

Dr. Rohm found that the active components in dog faeces were proteases — enzymes that degrade proteins. He reasoned that extracts from animal organs that produce similar digestive enzymes might be used instead. Fortunately for the tannery workers Rohm's investigations proved successful and by 1905 pig and cow pancreases were providing a more pleasant and reliable source of enzymes. To produce and sell the enzyme preparation Röhm teamed up with businessman Otto Haas to form the company *Röhm & Haas* in 1907. The pancreatic enzyme extract, which the firm called *Oropon®*, quickly became popular in the leather industry and *Röhm & Hass* followed this success with a range of other enzyme products.

World's first

Röhm continued his experiments with animal enzymes and in 1913 he was granted a German patent for the use of trypsin, extracted from animal pancreases, to clean soiled clothes. This is how Röhm explained his idea:

> *"As is known, tryptic enzymes have the ability to break down fat and protein. On the assumption that the main part of dirt in fabrics used by humans is composed of fat and protein residues, tryptic enzymes were added to the wash. It appeared that when this was done, the fabric could be cleaned in a shorter time, with less exertion of strength* and at a temperature far below the boiling point of water. Further, the fabric had a better appearance and much less soap was necessary. The advantage of using enzymes instead of other ingredients, especially alkaline chemicals, is that they do not attack the textile fibres..."*

In the same year that the patent was issued, *Röhm & Haas* started to market the world's first enzyme detergent, which they called *Burnus®*. Unfortunately the product was not a great success, and it is doubtful whether it worked very well. Its main components were washing soda

* *Röhm was writing at a time when most washing was done by hand and required physical strength.*

(sodium carbonate) plus a small amount of pancreatic extract. The washing soda made the solution very alkaline, and this would have reduced both the activity and stability of the enzymes. Röhm died in 1939, so it was left to others to develop the use of enzymes in washing products further.

Soap shortage

Major developments were next initiated in Switzerland by Dr. E. Jaag. Jaag had been inspired by Röhm's ideas when he started work in the 1930s and later by a wartime article proposing that enzymes could be used for cleaning clothes instead of soap. [During World War II, there was a severe shortage of fats (and consequently soap) in Europe, so enzyme-containing washing products could have assumed greater importance.]

However, Jaag had to wait until the war was over before he could make further progress, since all animal pancreases at the time were reserved for the production of insulin for treating diabetes.

Jaag's first product, *Bio38*, contained pancreatic trypsin with added bile salts to emulsify any fats. In 1947, he pointed out the advantages of using an enzyme product, but was also careful to indicate the potential disadvantages of using it to wash proteinaceous textiles *e.g.*, wool and silk. Jaag also noted that if such products were to have a future, new enzymes would have to be found that were tolerant of the alkaline conditions found in washing solutions.

Bacterial proteases

During World War II, much effort had been directed towards growing microorganisms in bulk. The fungus that makes penicillin requires oxygen for its growth, and initial attempts to produce penicillin for medical use had been based on surface culture in thousands of small containers including, at one time, bed-pans and milk bottles. A major advance was heralded by the development of fermenters in which the microbes were submerged in liquid medium and aerated with bubbles. These fermenters (sometimes referred to as Continuous Stirred Tank Reactors) allowed the industrial-scale production of microorganisms, for antibiotic production and subsequently the cultivation of microbes for enzyme production.

In 1959, Jaag, in collaboration with the *Swiss Ferment Company*, launched an improved product, *Bio40*. This contained a bacterial protease which worked best under neutral conditions. Although this was better than pancreatic enzymes, it was still not an ideal, since its stability in the alkaline conditions of the typical wash (pH 9–10) was too low.

While this was taking place, independent research in Copenhagen by *Novo Industri* (now *Novozymes*) was begun. The Danes' aim was to develop an enzymatic treatment for heavily-soiled overalls from the fish processing industry. Not only were the stains difficult to remove, but fishy odours emanated from the freshly-cleaned clothes as they were being ironed. By 1961 *Novo* had succeeded in producing a bacterial protease from *Bacillus licheniformis* that remained active and stable in alkaline washing conditions. Consequently the enzyme was given the trade name *Alcalase®*.

The associated washing treatment, a six-step procedure, required that the protein stains on the clothes were denatured in very hot (85 °C) detergent solution before the enzyme was added. Although the method gave excellent results compared with those normally used in industrial laundries, it was too complex to be widely adopted.

Confident in the knowledge that they had a better product than the *Swiss Ferment Company's* neutral protease, *Novo* approached Jaag's company, *Gebrüder Schnyder*, and signed an agreement to incorporate *Alcalase®* into *Bio40*. However, local competition from soap manufacturers prevented *Bio40* from becoming a success, so *Novo* and *Schnyder* entered into collaboration with a Dutch and a Danish company to produce and market an enzyme powder in Holland. In 1963 *Bio-tex* was launched, and within a short time it had captured 20% of the Dutch washing powder market.

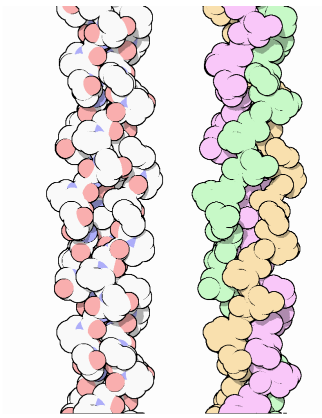
Since the introduction of optical brighteners into washing powders in the 1950s, the detergent business had remained more or less static. The sudden loss of a substantial proportion of the market to a newcomer aroused the attention of the major detergent manufacturers, who began to look at enzymes with renewed interest. Fifty years after Röhm's original patent, enzyme washing powders began to take off.

Today enzymatic detergents occupy 85% of the washing powder market in Western Europe and 50% of the market worldwide. Biological washing powders account for almost a quarter of the enzymes sold throughout the world. Most European consumers regard enzymes as beneficial and 'green', largely because of their effectiveness at low temperatures.

About gelatine

Gelatine (also spelt 'gelatin') is obtained from animals: usually pigs, cows or fish. Free gelatine molecules don't exist in meat or fish however. Gelatine is a breakdown product of a fibrous protein called collagen. Collagen gives mechanical strength to muscles, tendons and skin. When combined with minerals, it forms bones and teeth. Between 25% and 35% of the protein in a mammal's body is collagen.

Each collagen molecule is formed from three polypeptide strands bound together in a triple helix. Gelatine is made by partially breaking down collagen. When collagen is heated to approximately 60 °C, the strands of the triple helix start to separate. Further heating may cause the strands to break up into shorter polypeptides. When the solution is cooled down, the strands do not reform into collagen, but form a mass of tangled polypeptide chains, trapping water inside. Gelatine gel, with added sugar and flavouring, is familiar as a dessert and in confectionary.



A small portion of collagen, coloured by atom (left) and coloured to highlight the three chains (right). From: David Goodsell. April 2000. Molecule of the month: Collagen. doi: 10.2210/rcsb_pdb/mom_2000_4.