# Catalytic Decomposition of Urea by the Enzyme Urease

### Equipment:

3 goblets (conical glass cups) 3 glass rods beaker (10 mL) 2 measuring cylinder 2 dropping pipettes (optional: beaker (400 ml) hand blender and suitable plastic container funnel with filter paper and Erlenmeyer flask or better Büchner funnel with filter paper, suction flask and water aspirator or similar)



# Chemicals:

urea n-methylurea urease phenolphthalein indicator solution demineralized water (optional: soya beans)

## Safety:

urease:



H315, H319, H334, H335 P261, P264, P271, P302+352, P304+340+312, P305+351+338

phenolphthalein solution  $(C_{12}H_{14}O_4)$  (in ethanol):



H225, H319, H341, H350 P210, P280, P305+351+338, P308+313

Since both substances can cause eye irritation and urease can additionally cause skin irritation, it is essential to wear safety goggles and suitable protective gloves. In addition, urease can cause allergic reactions, asthma-like symptoms or breathing difficulties when inhaled, which is why inhalation must be avoided. Also, the experiment should be carried out in a fume hood. The soya bean extract may also lead to allergic reactions in rare cases (approx. 1 % of the population).

## Procedure:

<u>Preparation of the soya bean extract [1]:</u> The soya beans are soaked in a beaker overnight in about three times the amount of water. The soaked beans are then pureed together with about 10 mL of water per gram of dry soya beans (the water from soaking the beans can be used for this purpose) using a hand blender until a smooth mixture is obtained. Subsequently, the soya bean puree is filtered through filter paper in a funnel and the filtrate, which contains urease, is collected.

<u>Preparation</u>: A urea solution (w = 2 %) and an n-methylurea solution (w = 2 %) are prepared. By mixing 2 spatula- tips of urease with 3 mL of water in the 10 ml beaker, a urease suspension is obtained.

<u>Procedure:</u> Two of the three goblets are each filled with 200 mL of the urea solution, while the third is filled with 200 mL of the n-methylurea solution. Approximately the same amount of phenolphthalein indicator solution is added to all three solutions. The urea solution in the first goblet serves as a reference. A little bit of the urease suspension (or alternatively the urease-containing filtrate) is added to the urea solution in the second and the n-methylurea solution in the third goblet.

## **Observation:**

In the case of the urea solution in the second goblet, an increasing purple color is observed after a short time, while the n-methylurea solution remains unchanged.

#### Explanation:

The enzyme urease catalyzes the hydrolysis of urea, producing ammonia and carbon dioxide:

 $(NH_2)_2CO|w + H_2O|I \rightarrow CO_2|w + 2 NH_3|w$ .

An alkaline milieu forms due to the ammonia:

$$2 \text{ NH}_3|w + \text{CO}_2|w + 2 \text{ H}_2\text{O}|I \rightarrow 2 \text{ NH}_4^+|w + \text{HCO}_3^-|w + \text{OH}^-|w$$
.

For this reason, the change in color of the indicator phenolphthalein can serve to verify the hydrolysis. Structurally related substances like n-methylurea are, by contrast, not decomposed. This is an indication of the high substrate specificity of urease.

#### Disposal:

The solutions can be added to the waste water.

#### **References:**

[1] A. Lorenc: "Investigating the Action of Urea", Science in School, 2008, Vol. 9, pp. 39-44