

Summary of Results from
Group 6
Lactase Downstream Processing

Evreux 23. – 27.03.2026

Group 6 + presentation of group members

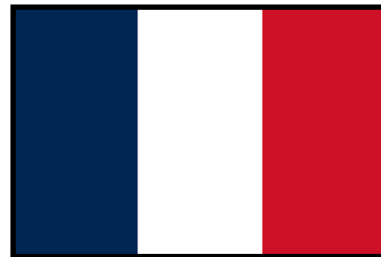
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Evaluation Steps of our Experiment

1. **PARTICLE FREE LYSATE – o-NPG-test (enzymatic activity assay)**
2. ANION EXCHANGE CHROMATOGRAPHY (AEXC) – o-NPG + photometer (enzymatic activity assay)
3. SDS – PAGE – gel + calibration line (purity, molecular weight – lactase)
4. RAW MILK TEST – colour change (conc. of glucose)

O-NPG-TEST of 1:10 diluted Particle Free Lysate

Test 50 μL supernatant + 500 μL o-NPG-solution.

Observation: Yellow colour.

Evaluation: $\beta(1\rightarrow4)$ glycosidic bond was broken.

Conclusion: Lactase is present and active.

→ **We can start AEXC.**



Evaluation Steps of our Experiment

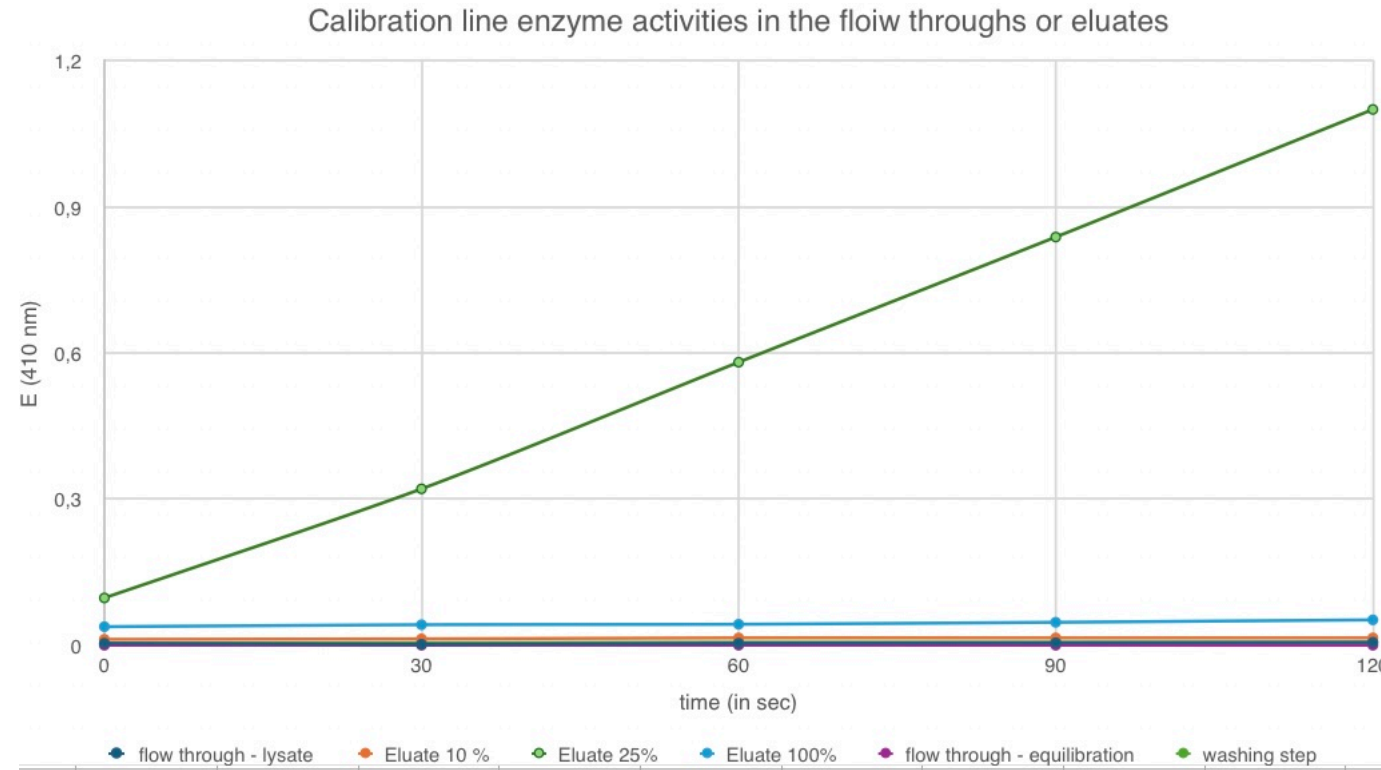
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5. EVALUATION

ANION EXCHANGE CHROMATOGRAPHY

O-NPG-test measured by photometer – enzymatic activity measurement

Test 50 μL eluate + 800 μL o-NPG-solution.

- **Observation:** Enzyme activity increases.
- **Evaluation:** Lactase is most active in the 25% eluate.
- **Conclusion:** Separation was successful.



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4. RAW MILK TEST – colour change (conc. of glucose)

SDS - PAGE

MARKER

S1: lysate (Diluted)

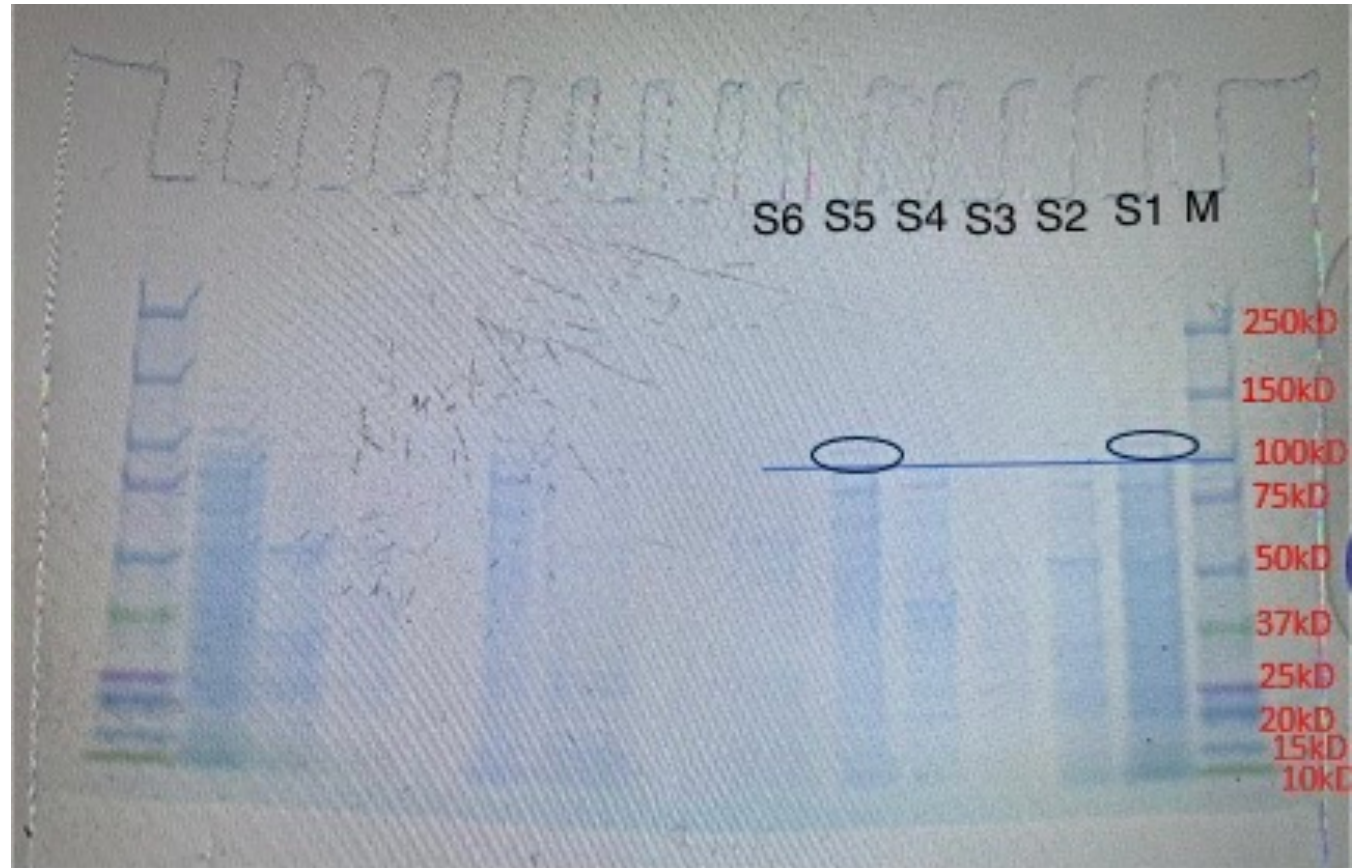
S2: loading flow through

S3: washing step

S4: eluate 10%

S5: eluate 25%

S6: eluate 100%



SDS - PAGE

Observation:

- Multiple bands are visible in the gel
- the marker indicates the known sizes in kDa (107)

Conclusion: Protein number reduced from ~3000 by AEXC, exact number unknown

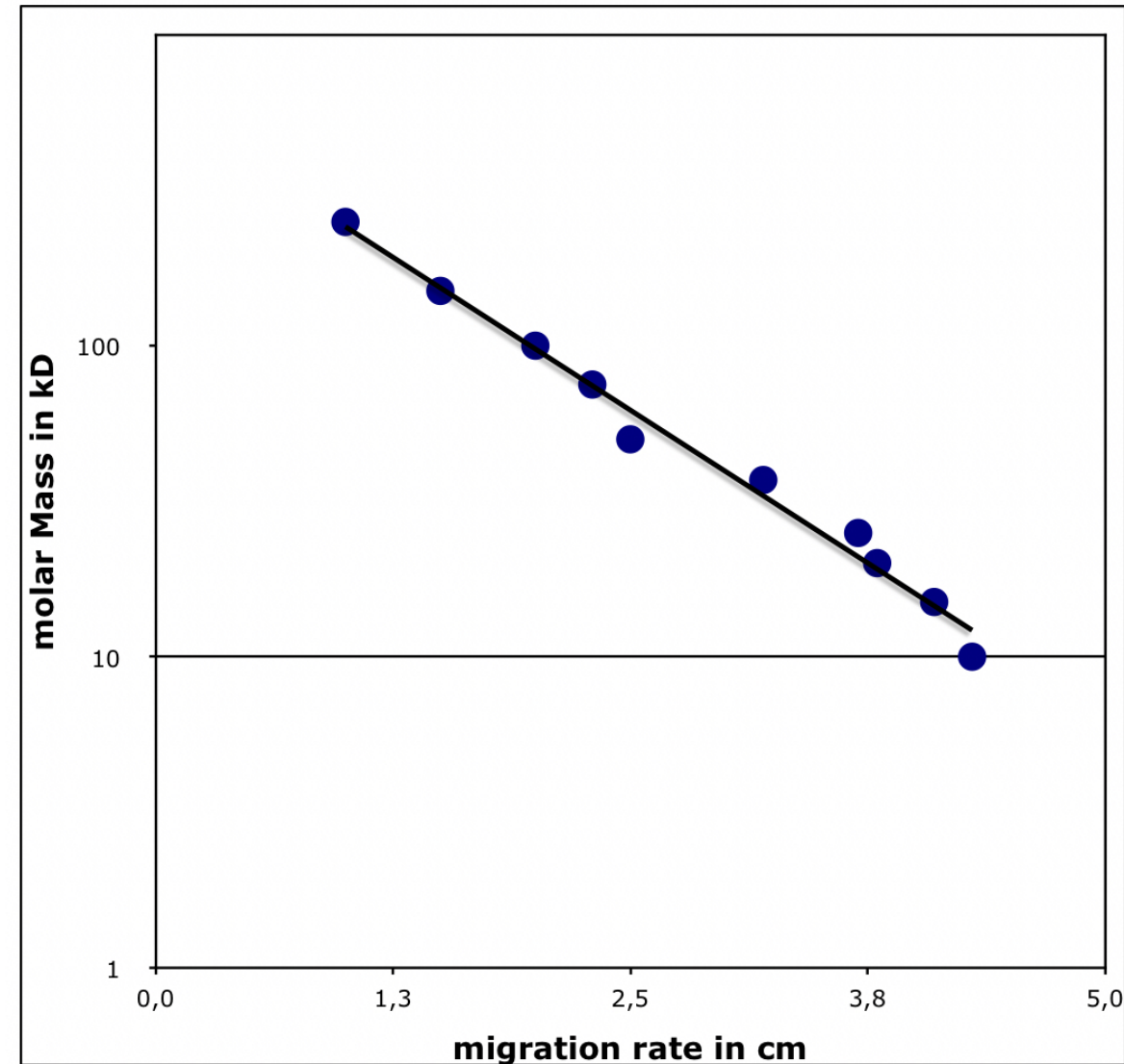


Evaluation of the SDS – PAGE

The molecular weight of lactase was determined using the calibration curve.

Migration rate: 1,9cm

Molecular weight: 107 kDa

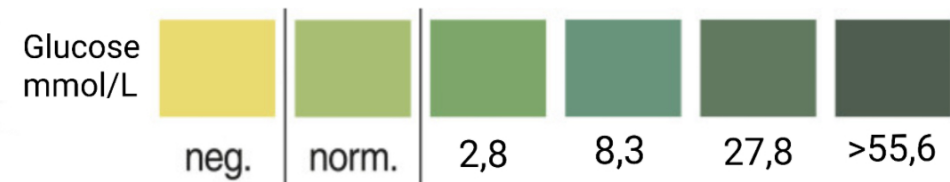
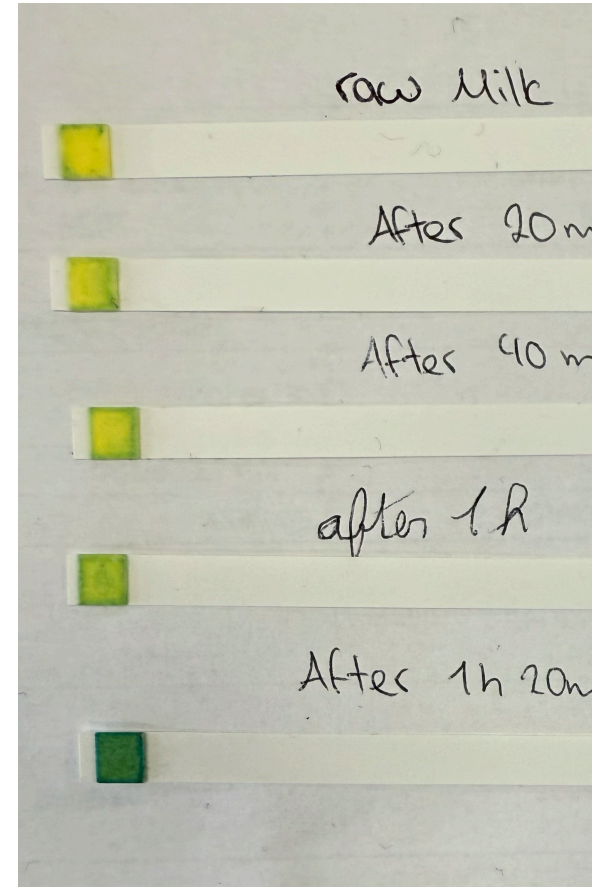


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4. **RAW MILK TEST – colour change (conc. of glucose)**

RAW MILK TEST

- **Observation**
- Colour changes from yellow to green over time.
- Colour becomes darker → increasing glucose concentration.
- **Evaluation:** After adding lactase, the reaction became faster, which can be seen in the stronger colour change.
- **Conclusion:** In conclusion, lactase breaks down lactose into glucose and galactose and speeds up the reaction.



Thank you for your
attention!