Production of sugars from silage

Ruohosta proteiinia – Final seminar

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Sugars from silage ?

- Silage contains usually ca. 50 % or more insoluble cell wall polysaccharides (cellulose and hemicellulose) and a small amount of free sugars
  → These sugars can be used as a raw material for fermentation processes
- Cell wall polysaccharides need to be released by hydrolysis – for grass, enzyme hydrolysis is an obvious choice
- Only part of the polysaccharides can be released directly by enzymes. Pretreatment is needed to recover them with high yield.
- Technical questions:
  - Which is the optimal pre-treatment (method, conditions, technical performance vs. cost)?
  - Enzyme cost should be minimized for economic reasons → optimal mix and dosage?
  - Yields?
  - Side products, e.g. fermentation inhibitors released or formed in pre-treatment?
  - What happens to protein?
- Based on earlier literature, pre-treatment conditions usable to corn stover or straw would roughly suit silage fibre as well




Composition of water extracted silage (= “silage fibre”)

With dry matter 18,3 % (a project sample)

- *Carbohydrates* — *58 % of d.m.*
  - *Cellulose* *36 %*
  - *Hemicellulose* *19 %*
  - *Pectin* *3 %*

This is a potential source of sugars in fermentation e.g. for protein production

- Protein — 9,3 % of d.m. (6,25 * N/Kjeldahl)
- Klason lignin — 17,1 % of d.m (acid insoluble)
  2,4 % of d.m (acid soluble)
- Ash — 5,5 % of d.m
- Extractives (heptane) — 6,9 % of d.m

(Totals 99 %)

Work done at VTT in the "Ruoho-project"

- The aim of the work was to produce maximum amount of sugars by enzyme hydrolysis from cell wall polysaccharides of silage, by:
  - Studying the need and effect of pre-treatments for enhancing hydrolysis
  - Preliminary optimization of pre-treatment conditions
  - Optimization of mixtures of commercial enzyme products
- Literature information and previous experience at VTT (on e.g. straw, reed canary grass) was used as basis for planning the work
- Selected pre-treatment techniques studied for silage from MTT:
  - Steam explosion, carried out in a 10 L bench-top reactor
  - Pretreatment using gaseous ammonia ("3G AFEX"), in a 5L pressure reactor
  - Treatment with 15 % ammonia water in a pilot scale extraction reactor
  - The effects of treatments were followed by:
    - Standard test for enzymatic hydrolysability
    - Mass balances
    - Carbohydrate analyses and balances
- Optimal enzyme mix was developed for two pre-treated materials
- Finally, a large amount of hydrolysate was produced at VTT Rajamäki pilot plant

Silage after ammonia water treatment (90°C, 20h)

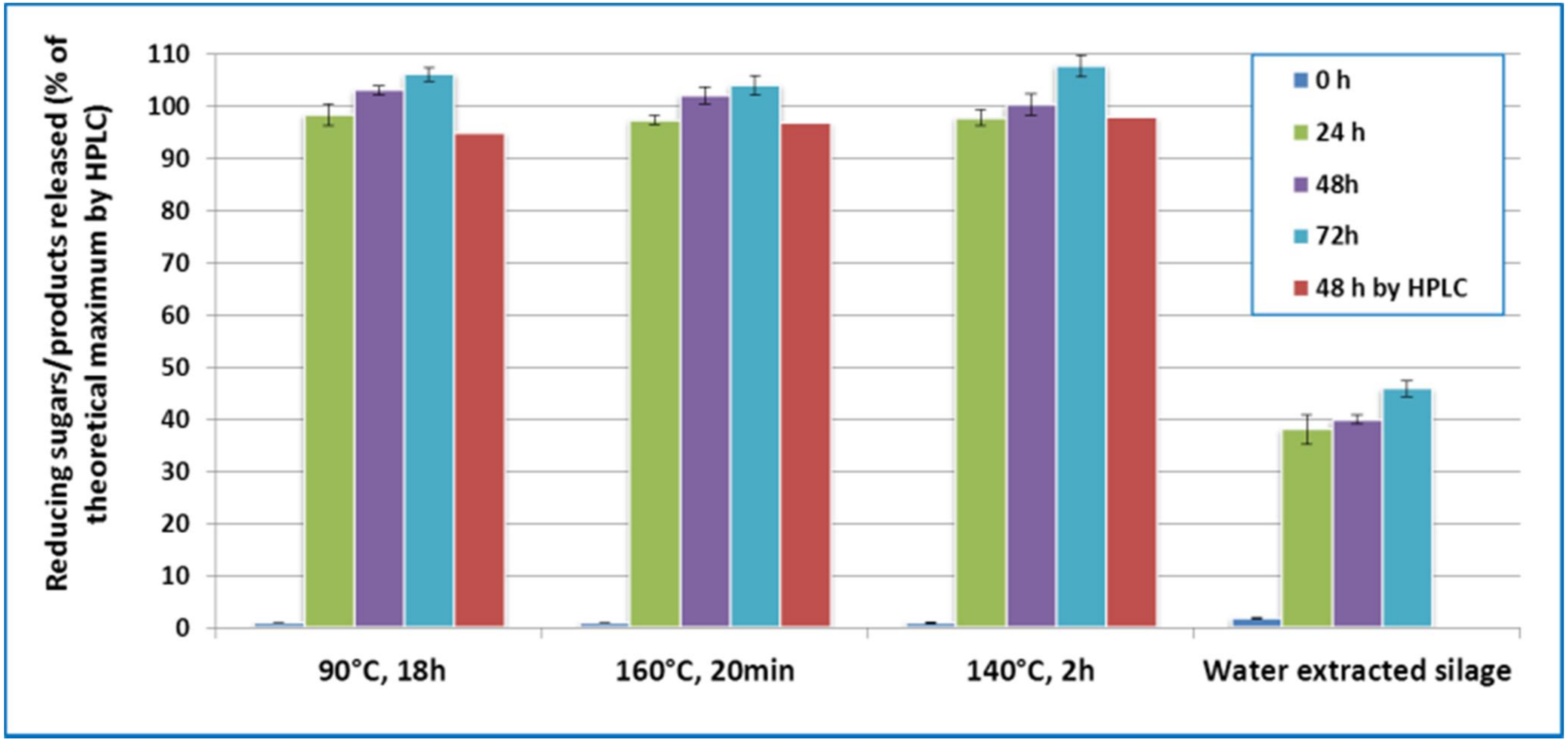
Original sample

Directly from the reactor

Water insoluble solids

Enzymatic hydrolysability of the materials after extraction with 15% ammonia water

(With commercial cellulase 10 FPU/g d.m. + β-glucosidase 100 nkat/g)



The yields as reducing compounds were still slightly overestimated but HPLC analysis (glucose+ xylose + arabinose after 48 h:n hydrolysis) confirmed almost complete hydrolysability. Acidic sugars from pectin (ca. 3 % of the raw material) were not analysed.

Silage after steam explosion

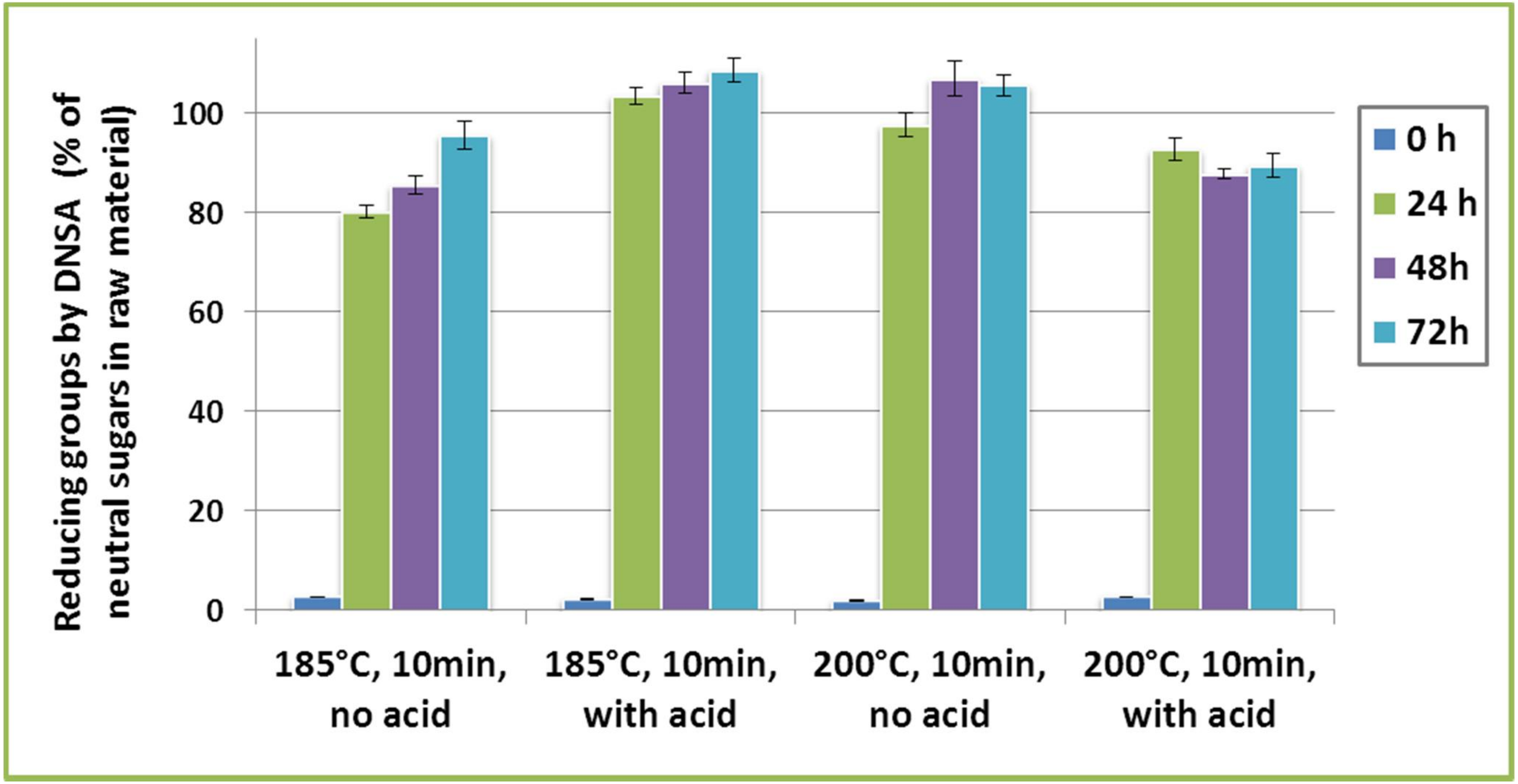
Untreated silage

185 °C, 10min (with acid)

200 °C, 10min (no acid)

Enzymatic hydrolysability of the steam exploded materials

(With commercial cellulase 10 FPU/g d.m. + β-glucosidase 100 nkat/g)

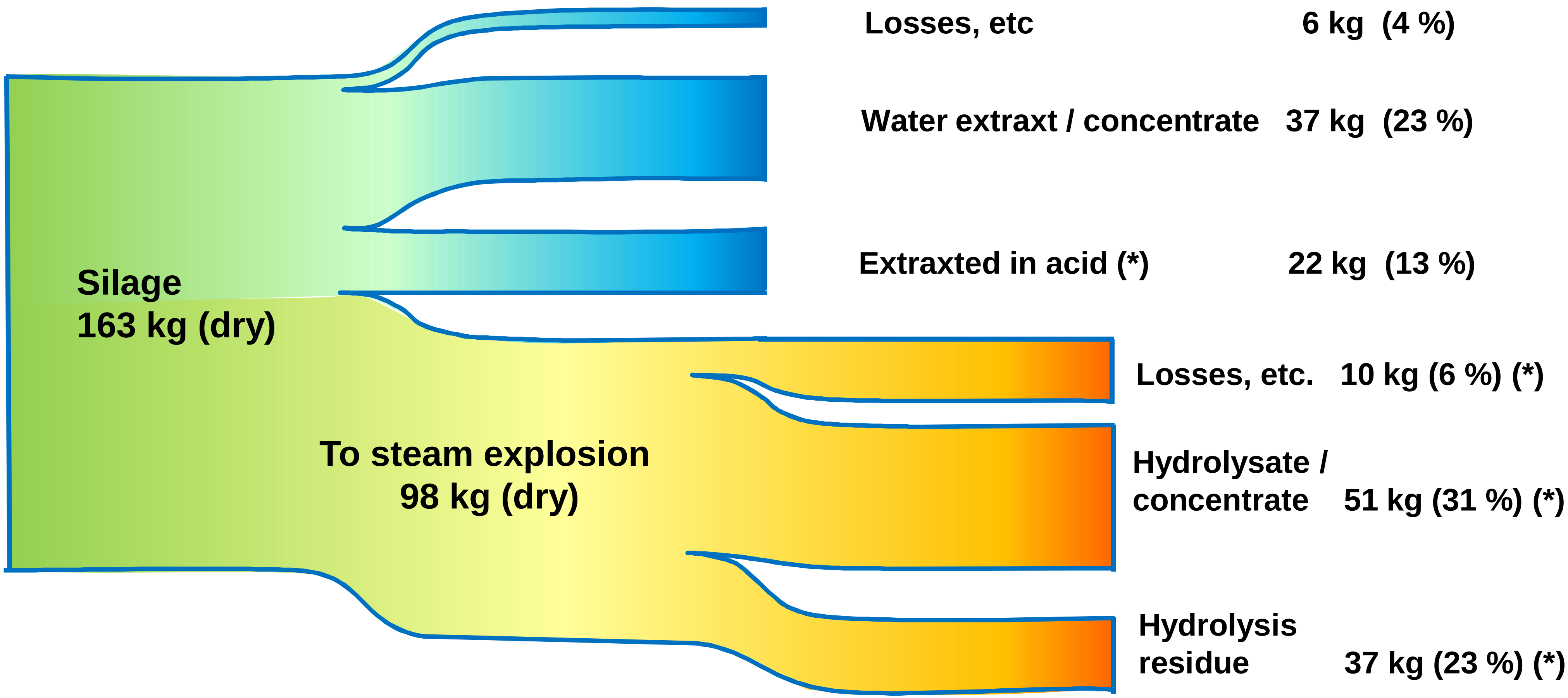


NOTE: Acidic sugars from pectin (ca. 3 % of the raw material) were not analysed

Production of large batch of hydrolysate in VTT Rajamäki pilot

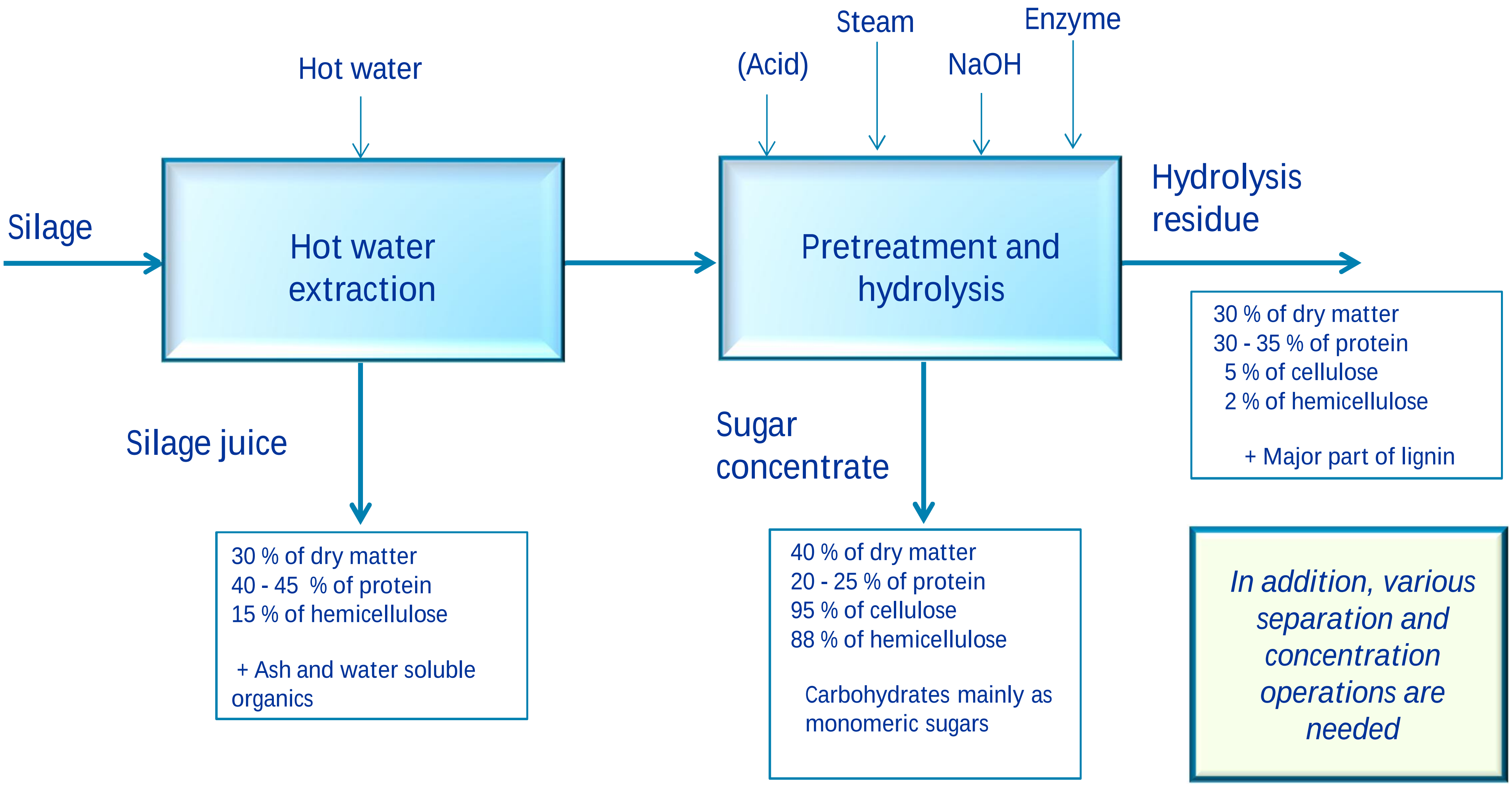
- The raw material was frozen silage from MTT (total ca. 750 kg)
- Hot (55°C) water extraction in a container with a sieved liquid outlet
  - → The extract containing free sugars and protein was concentrated by a vacuum film evaporator to ca. 77 kg of sugar solution (with 37 kg d.m.)
- The extracted silage was acidified by flushing using 1,5 % phosphoric acid, the used acid was discarded
- The acidified silage was steam exploded in batches, two batches at ca. 165 -175 °C (with holding time 20 -30 min)
- Steam exploded slurry was hydrolyzed using a commercial cellulase (10 mg protein /g d.m) and pectinase (0,5 mg protein/g d.m.) at pH 4,8, 50 °C for ca. 92h
- The solid residue was separated using centrifugation
  - → The hydrolysate was concentrated by evaporation, resulting in ca. 100 kg of hydrolysis sugar solution (with 51 kg d.m.)
  - → Sugar concentrate to Aalto University

Pilot run, mass balances (dry matter)



(*) The amounts do not count for added phosphoric acid, enzymes and salts from neutralization before hydrolysis, counted as NaH_2PO_4 to be 14,8 kg

Simplified schematic process for sugar production (how it could be)



Conclusions

- Silage after hot water extraction contains more than 50 % of cell wall carbohydrates and is a potential raw material of further fermentation for e.g. protein production.
- For efficient hydrolysis by economic enzyme dosage, pre-treatment is required:
  - Steam explosion proved to be a functional option. Advantages: Well established and simple method. Main disadvantage: Loss of hemicellulose
  - Treatment with gaseous ammonia produced a well-hydrolysable fibre but proved to be problematic due to challenging operations with gaseous ammonia
  - Treatment with 15 % ammonia water worked technically well and produced very well hydrolysable material. Technical challenges: recovery of ammonia and dealing with lignin which dissolved together with protein to ammonia water.

  → In general rather mild conditions (as compared to other biomasses) are sufficient for pre-treatment of silage
- The process was scaled up to small pilot scale with steam explosion, followed by hydrolysis using moderate enzyme dosage, leading to hydrolysis degree of ca. 90 %
- Further simplification of the process and more detailed optimization is needed for feasible process.

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