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Fermentation of the fractions from silage processing

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Fractions studied

1. Silage juice
   - Applicability as a replacer for high-value, complex components in growth media for industrially relevant microbes (University of Oulu / Laboratory of bioprocess engineering)

2. Hydrolyzate from extracted silage
   - Feed protein production in Pekilo-fermentation
   - Applicability of the hydrolyzate as C/energy source for Pekilo
   - Requirements of supplementary nutrients in fermentation
   - Techno-economic parameters for the fermentation process

Silage juice as a component in growth media

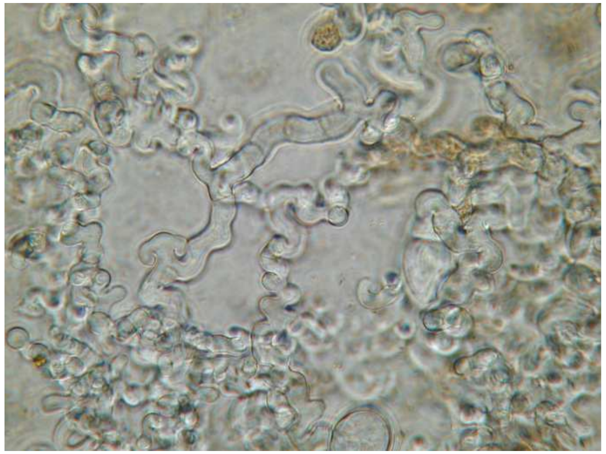
1. Inhibitory effects of weak organic acids (lactic/formic/acetic acids)
    - *E.coli* and *Bacillus subtilis* : 100 % inhibition of growth with 50 % juice concentration (replacing water)
    - *L.rhamnosus, S.cerevisiae* and *Pichia pastoris*: growth and glucose consumption slow down and cellmass yield decreases by 25…50 %

2. Nutrients in the juice
    - *L.rhamnosus, S.cerevisiae* and *P.pastoris* grow on the juice without any supplementary nutrients (peptone and yeast extract)
    - The addition of peptone and/or yeast extract into the juice have no effect on growth, rate of glucose consumption or cellmass yield

=> Silage produced with enzyme treatment probably would produce juice applicable as a nutrient source in media for growing microbes

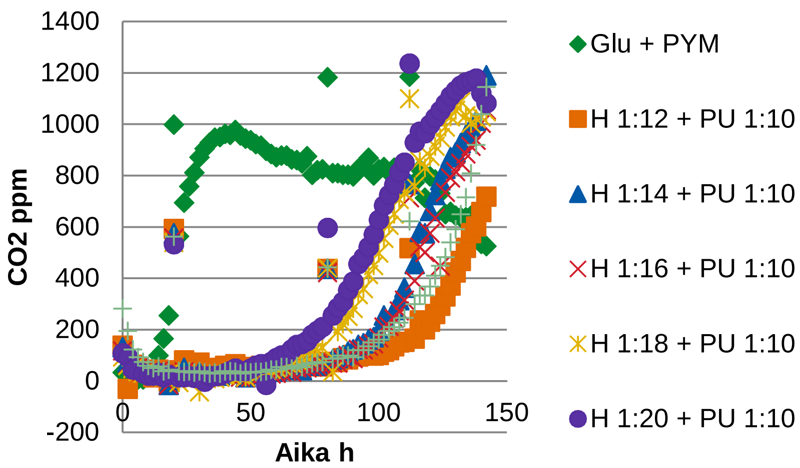
Production of feed protein on silage hydrolyzate

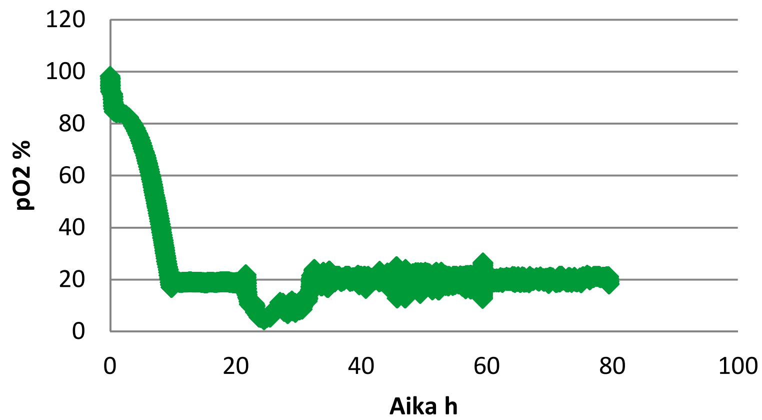
1. Why Pekilo (*Paecilomyces variotii*)?
   - Hydrolytic fungus
   - Tolerates inhibitors in hydrolyzates better than e.g. yeasts
   - Is even able to use furfural and HMF as C/energy source
   - A filamentous organism => easy to filtrate out from the broth to high dry substance (30 % +)
   - Long history of use as SCP-product and approved feed component
   - Protein content 50 – 60 %



Production of feed protein on silage hydrolyzate

1. Inhibitory effect of the process fractions
   - Hydrolyzate/extract –combination increases remarkably lag-time
   - The effect can be avoided by adaptation to the fractions (→ hydrolyzate 1:5 = appr. 100 g/L glucose)

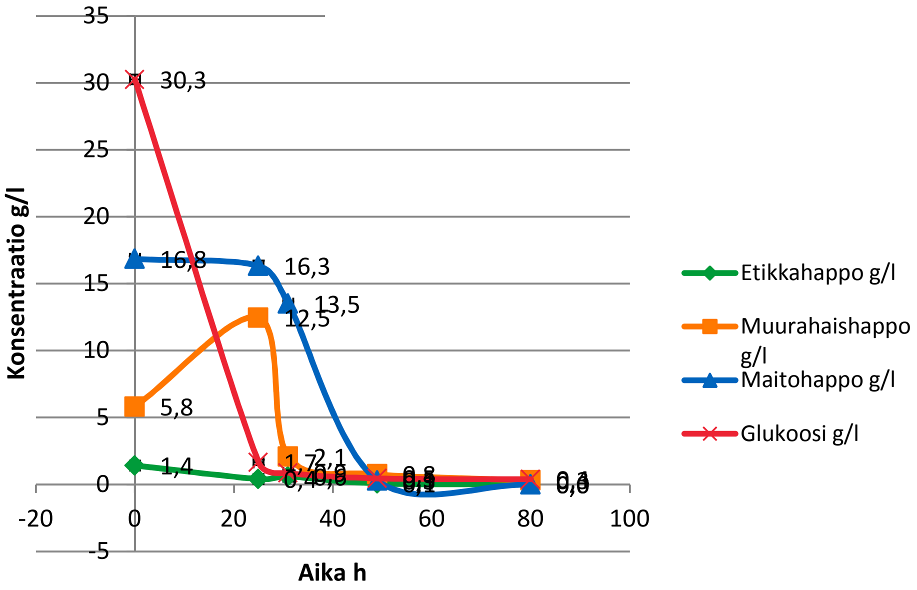


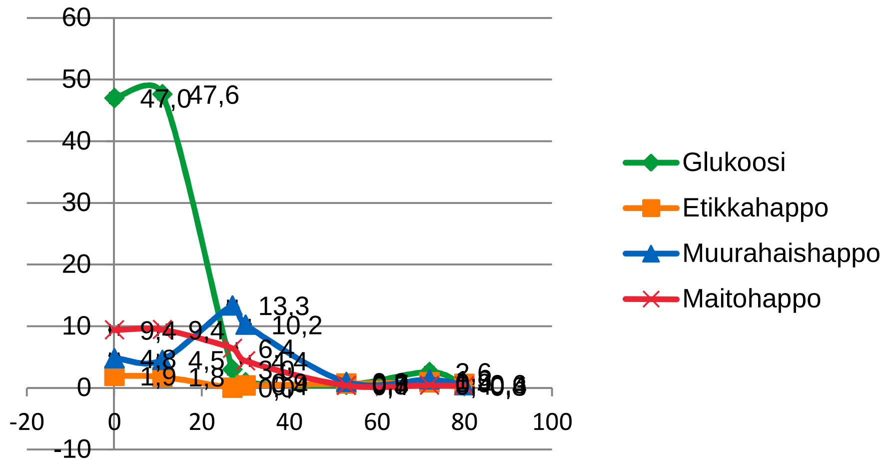


Production of feed protein on silage hydrolyzate

2. Consumption of glucose and acids in fermentation

- In a fed-batch fermentation the final cell dry weight concentration was 37 g/L and cellmass yiedl on glucose was 0,49 g/g (0,49 – 0,67 g/g)





Production of feed protein on silage hydrolyzate

3. Need for supplementary nutrients
   - The xtract can be replaced with e.g. CSL (5 g/25 g glucose) and diammoniumhydrogen phosphate (5 g/25 g glucose)

4. Specific growth rate (μ = dX/(X dt))
   - Based on fed-batch experiments μ → 0,16 h^{-1}
   - Continuous chemostat cultivation experiments done μ → 0,12 h^{-1} with max. glucose concentration 30 g/L limited by the oxygen transfer capacity of the fermentor and practical arrangements in small-scale (appr. 2-fold value achievable in large-scale meaning volumetric cellmass productivity appr. 3,7 g/Lh]

5. Asepticity
   - Continuous fermentation was run > 500 h without any contamination

Production of feed protein on silage hydrolyzate

6. Techno-economic parameters

- Continuous fermentation; residence time ≥ 6 h
- Cellmass volumetric productivity 3,7 g/Lh
- Cellmass yield on glucose 0,5 g/g
- Cellmass yield on oxygen 0,85 g/g
- Working volume of the fermentor 80 m^3
- Annual productivity of dry Pekilo 2400 t
- CSL and diammoniumhydrogen phosphate consumption appr. 500 t/a
- Power consumption in fermentation 5 000 MWh/a

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