L8: Membrane bioreactor –
A new approach for enhancing main fermentation velocities

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Lisa Stumpf, Nicolas Werner, Stefan Schildbach

Hochschule Fulda - University of Applied Sciences
Faculty Food Technology, Institute for Bioprocess Engineering
Fulda, Germany
## Continuous main fermentation

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process time decreases → increase in time-space-yield</td>
<td>Less flexibility concerning different beer types and output variations</td>
</tr>
<tr>
<td>Smaller coldblock → lower invest</td>
<td>Even raw material quality required for securing process stability, particle-free wort</td>
</tr>
<tr>
<td>Decrease in specific energy- and utility-consumption, avoiding peak consumption, decrease in extract losses</td>
<td>Changes in beer quality in respect to higher alcohols, esters, organic acids, sulphur-compounds → deviations in smell and taste</td>
</tr>
<tr>
<td>Increase in productivity</td>
<td>Vulnerable towards microbial contamination</td>
</tr>
<tr>
<td>Lower operating costs, e.g. for cleaning and disinfection</td>
<td>Long start-up phase</td>
</tr>
<tr>
<td></td>
<td>Implementation between batch processes is difficult to realize (e.g. wort storage)</td>
</tr>
<tr>
<td></td>
<td>Higher effort in machinery, control strategy and personnel</td>
</tr>
</tbody>
</table>

(Annemüller and Manger 2009, modified)
Target: Continuous beer production

- Target(s):
  
  - Continuous main beer fermentation as part of an overall continuous beer production

  - Decrease in main fermentation time from approx. 3 – 4 days to less than 1 day

1: Wort filter
2: Wort buffer tank
3: Kräusenbottich
4: Bioreactor
5: Maturation tank
6: Storage tank

Some information about the bioreactor:

- Frame filter, containing a mixture of yeast and kieselguhr
- Thickness yeast layer: 25 – 30 mm
- Flow rate: 1 – 1.5 cm/h \(\rightarrow\) retention time approx. 2 h!
- Production: 15 l beer \(/(m^2*h)\)

Background: Different reactors continuously operated

- Fixed bed reactor
- CSTR operated continuously (Chemostat)
Background: Batchfermentation scheme

$X = \text{Biomass concentration}$
$c_S = \text{Substrate}$
$c_P = \text{Product}$
(dotted line: secondary metabolites)

Phase 1: Lag-phase
Phase 2: Exponential growth phase
Phase 3: Transition phase
Phase 4: Stationary phase
Phase 5: Mortality phase
Background: Monod kinetic (growth limited by substrate concentration)

Growth limited by substrate concentration:

Max. growth rate $\mu_{\text{max}} = 0.5 \, 1/h$

Monod constant $k_S = 0.1 \, \text{g/l}$

Monod kinetic:

$$\mu (c_S) = \mu_{\text{max}} \times \frac{c_S}{k_S + c_S}$$

With $k_S = \text{Monod constant}$
Background: Different reactor types

- Fixed bed reactor
- Mixed bed reactor
- Gaslift reactor
- Module loop reactor

(taken from Ludwig 2003)
### Background:
Different reactor types

<table>
<thead>
<tr>
<th>Batch process</th>
<th>Continuous processes with immobilized yeast</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up-to-date technology</td>
<td>Fixed bed reactor</td>
<td>19*</td>
</tr>
<tr>
<td></td>
<td>Mixed bed reactor</td>
<td>29.5*</td>
</tr>
<tr>
<td></td>
<td>Module loop reactor</td>
<td>55*</td>
</tr>
<tr>
<td>Process time (h)</td>
<td>approx. 96</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Dilution rate (1/h)</td>
<td>0.010</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>0.034</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.050</td>
<td></td>
</tr>
</tbody>
</table>

(values taken from Ludwig 2003 (assumed to be conservative))
Background: Batchfermentation

1: total extract / gravity
2: Sugars
3: pH
4: Oxygen, dissolved
5: osmotic pressure
6: Yeast concentration
7: Ethanol concentration
8: Carbon dioxide at constant pressure

(Annemüller et al. 2004)
Background: Batchfermentation

Phase I: Crabtree (=Substrateinhibition)

Phase II: Crabtree + Productinhibition

Phase III: Productinhibition + Substratelimitation

(Annemüller et al. 2004)
Background: Physiology of the yeast

Figures for Saccharomyces cerevisiae.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Yield $Y$ (g/g)</th>
<th>Max. growth rate $\mu_{\text{max}}$ (1/h)</th>
<th>Monod constant $k_S$ (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, aerob</td>
<td>0.56</td>
<td>0.50</td>
<td>0.1</td>
</tr>
<tr>
<td>Glucose, Crabtree</td>
<td>0.14</td>
<td>0.34</td>
<td>0.0</td>
</tr>
<tr>
<td>Glucose, anaerob</td>
<td>0.15</td>
<td>0.25</td>
<td>0.1</td>
</tr>
<tr>
<td>Ethanol, aerob</td>
<td>0.67</td>
<td>0.36</td>
<td>0.1</td>
</tr>
</tbody>
</table>

(Bodenschatz, taken from Hass and Pörtner 2009)
Background:
Product inhibition by ethanol

(Annemüller et al. 2004)
Background: Batchfermentation of beer

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University of Applied Sciences

(Annemüller et al. 2004)
Background:
Different fermenters in a row

(Branyik et al. 2008)
Background:

(Diagramm entnommen aus Ludwig 2003)

Aeration rate related to maximum (output controller)

Max. aeration rate

pH-value

(Figure taken from Ludwig 2003)
Approach: Membrane Bioreactor

• Membrane bioreactor allows retention of the yeast; no yeast immobilization necessary to achieve high concentrations for high fermentation velocities.

• Aeration can be adjusted in order to achieve an appropriate yeast growth (e.g. $60 \times 10^6$ cells/ml).

• Regular yeast harvest will keep the yeast concentration within a target range.

• Yeast concentration and age can be adjusted independantly from each other (ratio of aeration to yeast harvest) $\rightarrow$ new parameter

• $\rightarrow$ Appropriate beer quality compared to batch production at high fermentation velocities ($< 20$ h).
Approach: Comparison to conventional fermentation

- **Conventional**
  - Batch process
  - Takes approx. 3 days + set-up time for the next batch

- **MBR Beer**
  - Continuous process
  - Target is to cut down fermentation time to < 1 day
Approach:
Operation of MBR

Alternating inlet and outlet through the same membrane.
Approach: Operation of MBR

Outlet green beer

Inlet wort
Approach: Membrane „backwash“

Outlet green beer

Inlet wort
1. Does an increase in freely suspended yeast cells cut down fermentation time to < 20 h? How high is the yeast cell count necessary?

2. Is a continuous and stable operation possible?

3. Is it possible to achieve an appropriate beer quality by adjusting aeration and yeast harvest?

4. Can the membranes be kept free from blocking under these conditions whilst retaining an economic flux (e.g. 10 – 20 l/(m²*h)) for a long period of time? What kind of operation, backwash and cleaning regime is necessary?

5. …
## Approach: Yeast concentration (calculated values)

<table>
<thead>
<tr>
<th>Yeast cell count (*10^6 cells/ml)</th>
<th>Yeast dry matter (g/l)</th>
<th>Pitching rate (l/hl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>20</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>50</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>100</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>200</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>400</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>4000</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
## Background: Yeast concentration

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Conventional</th>
<th>Accelerated</th>
<th>Strongly accelerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitching rate</td>
<td>l/hl</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Yeast cell count after pitching</td>
<td>$10^6$ cells/ml</td>
<td>15</td>
<td>approx. 30</td>
</tr>
<tr>
<td>Fermentation time at 9 °C</td>
<td>days</td>
<td>8 - 9</td>
<td>6 - 7</td>
</tr>
<tr>
<td>Yeast cell count max.</td>
<td>$10^6$ cells/ml</td>
<td>50 - 60</td>
<td>70 - 80</td>
</tr>
<tr>
<td>Yeast harvest</td>
<td>l/hl</td>
<td>approx. 1.5 – 2</td>
<td>approx. 2 - 3</td>
</tr>
<tr>
<td>Exract decrease within first 24 h</td>
<td>% $E_a$</td>
<td>0.3 – 0.5</td>
<td>0.8 – 1.0</td>
</tr>
<tr>
<td>pH-drop within first 24 h</td>
<td>-</td>
<td>0.25 – 0.30</td>
<td>0.4 – 0.6</td>
</tr>
<tr>
<td>Temperature increase within first 24 h without cooling</td>
<td>K</td>
<td>0.5 – 1.0</td>
<td>1.4 – 2.0</td>
</tr>
</tbody>
</table>

(Annemüller et al. 2004)
First results:
Increase yeast cell count
First results: Increase yeast cell count

(Zeit (h)
ORP Zellzahl Alkohol Ew)

Zellzahl (Mio. Zellen/mL)
ORP (mV)
Alkohol (Vol.-%)
Ew (Gew.-%)

Alkohol (Vol.-%)
Ew (Gew.-%)

(Zeit (h)
ORP Zellzahl Alkohol Ew)

Yeast cell count increases with time.
First results:
Increase yeast cell count

Hefe-TS (g/L)

Zellzahl [Mio. Zellen/mL]

Probenvolumen/Probenvolumen (m/Vol-%)

Zellzahl [Mio. Zellen/mL]

(Stumpf 2014)
First results:
Increase yeast cell count

→ Increase in yeast cell count leads to higher fermentation velocities.

(Stumpf 2014)
First results: Increase yeast cell count
First results:
Increase yeast cell count

When exchanging $< 200 \text{ ml/h} (< 250 \text{ ml/h})$ the ethanol concentration stayed constant, which equals a fermentation time of $< 30 \text{ h} (< 24 \text{ h})$ (reactor volume used was 6.0 l).

Yeast cell count: approx. $330 \times 10^6$ cells/ml.

Transfer to a continuously operated is probable.
<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Does an increase in freely suspended yeast cells cut down fermentation time to $&lt; 20$ h? How high is the yeast cell count necessary?</td>
<td>(yes)</td>
</tr>
<tr>
<td>2.</td>
<td>Is a continuous and stable operation possible?</td>
<td>(yes)</td>
</tr>
<tr>
<td>3.</td>
<td>Is it possible to achieve an appropriate beer quality by adjusting aeration and yeast harvest?</td>
<td>?</td>
</tr>
<tr>
<td>4.</td>
<td>Can the membranes be kept free from blocking under these conditions whilst retaining an economic flux (e.g. $10 – 20$ l/(m$^2*h)) for a long period of time? What kind of operation, backwash and cleaning regime is necessary?</td>
<td>?</td>
</tr>
<tr>
<td>5.</td>
<td>…</td>
<td></td>
</tr>
</tbody>
</table>
Literature


Literature

- Stumpf, Lisa: Beschleunigung der Hauptgärung bei der Bierbereitung durch gezielte Einstellung der Hefekonzentration (Increase in Main Fermentation Velocity by Adjustment of the Yeast Cell Count). Masterarbeit, Hochschule Fulda University of Applied Sciences, Fulda, 2014

- Werner, Nicolas: Versuche zur Entwicklung eines Verfahrens zur quasikontinuierlichen Hauptgärung der Bierbrauerei mit Hilfe eines Membran-Bioreaktors (Tests using a Membrane Bioreactor for the Main Fermentation of Beer). Masterarbeit, Hochschule Fulda University of Applied Sciences, Fulda, 2014
Weiterführende Literatur

<table>
<thead>
<tr>
<th>Werkstoff</th>
<th>nach Fa. Pall</th>
<th>nach Fa. Filtrox</th>
</tr>
</thead>
<tbody>
<tr>
<td>mittl. Porendurchmesser der</td>
<td>α-Al₂O₃</td>
<td></td>
</tr>
<tr>
<td>Membranschicht</td>
<td>0,8 µm</td>
<td>0,9 µm</td>
</tr>
<tr>
<td>Modullänge</td>
<td>1020 mm</td>
<td></td>
</tr>
<tr>
<td>Kanaldurchmesser</td>
<td>6 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>Anzahl der Kanäle/Element</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Memranfläche/Element bei 6 mm Ø</td>
<td>0,36 m²</td>
<td>beig 8 mm Ø: 9,1 m²</td>
</tr>
<tr>
<td>Anzahl der Elemente/Modul bei 6 mm Ø</td>
<td>22 ≈ 13,0 m²</td>
<td></td>
</tr>
<tr>
<td>Ø - Fließgeschwindigkeit in den Kanälen</td>
<td>1,5…2 m/s</td>
<td>3…≤ 5 m/s</td>
</tr>
<tr>
<td>max. HTS-Gehalt</td>
<td>&lt; 20 %</td>
<td>&lt; 20 %</td>
</tr>
<tr>
<td>Ø - Permeatdurchsatz (abhängig von HTS-Gehalt des Unfiltrats)</td>
<td>17…20 l/(m²·h)</td>
<td>15…25 l/(m²·h)</td>
</tr>
</tbody>
</table>

(Annemüller et al. 2004)
Permeatdurchsatz in hl/h

Transmembrandruck in bar

Hefekonzentration in %

Brauerei A, Brauerei B, Brauerei C, Brauerei D, A, B, C, D
Abbildung 96 Redoxreaktion des NAD/NADH + H⁺

Katabolismus

Anabolismus

(Annemüller et al. 2004)
Background:
Yeast metabolism

(Annemüller et al. 2004)
Background: Chemostat

Chemostat

(--> continuously operated)

- **Biomass X** (g/l)
- **cS** (g/l)
- **Dilution rate D** (normed at µ max)

Example:
- \( Y_{XS} = 0.56 \text{ g/g} \)  \( \mu_{\text{max}} = 0.5 \text{ 1/h} \)
- \( c_{S\text{ein}} = 1 \text{ g/l} \)  \( k_S = 0.1 \text{ g/l} \)
Vergleich Chemostat - Perfusion

(Hass and Pörtner 2009)