Biological, technical and economical feasibility of a rotifer recirculation system

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Rotifers are an excellent first food for fish larvae but...
...a bottleneck for mass culture and industrial applications

- unpredictable in production
- source of contamination
- variable in quality
- high maintenance cost

Batch cultures
Recirculation system

- Rotifer culture
- Protein skimmer
- Biofilter
- Settling tank

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Recirculation system

Hydrology and food distribution

- Mesh size of the filter
- Determination of the optimal flow rate
- Determination of the feeding regime
- Improvement of the diet

Water quality

- Improvement of physical water quality parameters
- Improvement of biological water quality
- Technical improvement (more performant equipment)

Commercial applications and rotifer quality

- Upscaling for commercial application
- Effect on rotifer quality
- Cost estimation
Determination of a mesh size that retains rotifers

Mesh 30 µ
Determination of the optimal flow rate
Determinant of the feeding regime

- Batch
- CS
- CS improved feeding regime

Density (ind./ml) vs. Day

Day: 0 1 2 3 4 5 6 7
Density: 0 2000 4000 6000 8000
Improvement of the diet

\[ \text{CSH} = 0.035D^{0.415V} \]

- **Batch**
- **CS**
- **CS improved feeding regime**
- **CSH**
- **CSH improved feeding regime**

**Graph:**
- Y-axis: density (ind./ml)
- X-axis: day (0 to 7)

**Legend:**
- Batch
- CS
- CS improved feeding regime
- CSH
- CSH improved feeding regime

**Equation:**
\[ \text{CSH} = 0.035D^{0.415V} \]
Recirculation system

✓ Hydrology and food distribution
  ✓ Mesh size of the filter
  ✓ Determination of the optimal flow rate
  ✓ Determination of the feeding regime
  ✓ Improvement of the diet

Water quality

• Improvement of physical water quality
• Improvement of biological water quality
• Technical improvement
Improvement of physical water quality parameters

Effect of ozone in the recirculation system?

- Strong oxydant
- Strong disinfectant
- Coagulation/floccul.

Ozone
Protein skimmer
500%.day⁻¹
Settling tank
Biofilter
Oxygen Reduction Potential (ORP)

- culture
- protein skimmer
- culture (control treatment)

ORP (mV)

- 1 2 3 4 5 6 7 8 9 10 11 12 13

Day
Effect of ozone on the production of rotifers

- **Ozone**
- **Control**

Density (individuals ml⁻¹) vs. Day

Day: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15

Density: 0, 2500, 5000, 7500, 10000, 12500, 15000, 17500, 20000, 22500, 25000, 27500, 30000, etc.
Performance of the protein skimmer

Control

Ozone
# Performance of the protein skimmer

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Ozone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent water (ml)*</td>
<td>730</td>
<td>2100</td>
</tr>
<tr>
<td>Dry weight of SS (gr)*</td>
<td>36</td>
<td>102</td>
</tr>
<tr>
<td>SS composition (particles/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- uneaten food</td>
<td>$1.1 \times 10^{11}$</td>
<td>$3.2 \times 10^{11}$</td>
</tr>
<tr>
<td>- organic wastes</td>
<td>$4.1 \times 10^{11}$</td>
<td>$5.7 \times 10^{12}$</td>
</tr>
</tbody>
</table>

* = average (10 days)
Physico-chemical parameters

NH$_4^+$ (mg/l)

- Ozone
- Control

Day
Physico-chemical parameters

NO$_2^-$ (mg/l)

day

ozone

control
Effect of ozone on the rotifer culture water

100 µm

flocules & debris  lorica

control

100 µm

flocules

ozone
Physico-chemical parameters

**NO$_3^-$ (mg/l)**

- **ozone**
- **control**

Day: 0 1 2 3 4 5 6 7 8 9 10 11 12 13
Physico-chemical parameters

Absorbance (600nm)

- Ozone
- Control

Day
### Bacterial counts on culture water (CFU ml⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ozone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marine agar</td>
<td>TCBS</td>
</tr>
<tr>
<td>Rotifer culture</td>
<td>9.0 × 10⁴</td>
<td>1.8 × 10³</td>
</tr>
<tr>
<td>Protein skimmer</td>
<td>1.0 × 10⁵</td>
<td>5.4 × 10²</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Beneficial effects of ozone:

✓ improved water quality
  - better removal of particles (oxidation, flocculation)
  - lower level of ammonium, nitrite and nitrate
    ⇒ possibility to reduce the size of the biofilter

✓ improved rotifer production
  - high rotifer density
  - stable and longer culture period
    ⇒ possibility to reduce size of rotifer tanks

✓ cleaner rotifer production
Improvement of the biological water quality
Effect of substrate of the biofilter on the growth rate of rotifers

rot./ml vs day

- gravel
- CaCO₃

0 1 2 3 4 5 6 7 8 9 10 11

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Technical improvement (more performant equipment)

Floc traps  Sintered metal filters
Sintered metal filters

Rotifer growth

![Graph showing rotifer growth comparison between nylon and sintered metal filters. The x-axis represents days, ranging from 0 to 9, and the y-axis represents density (ind.ml⁻¹), ranging from 0 to 8000.]
Sintered metal filters

Ammonia

![Graph showing ammonia levels over days with nylon filter and sintered metal filter comparison.](image-url)
Recirculation system

Hydrology and food distribution
- Determination of the optimal flow rate
- Determination of the feeding regime
- Improvement of the diet

Water quality
- Improvement of physical water quality parameters
- Improvement of biological water quality
- Technical improvement (more performant equipment)

Commercial applications and rotifer quality
- Upscaling for commercial application
- Effect on rotifer quality
- Cost estimation
✓ Upscaling for commercial application
Schematic outline of the upscaling system

1 = filter (30 µm)
2 = air water lift

Culture tank (1000 l)
Settling tank (100 l)
Biofilter (750 l, 350 l gravel, 100 l CaCO₃)
Protein skimmer
Protein skimmer (in series)
ozone

500%
Rotifer production in recirculation system

Density (individuals.ml⁻¹)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

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Rotifer production at 3000 ind./ml stocking density

![Graph showing rotifer production with harvesting marked on day 7. The y-axis represents density (ind.ml⁻¹) and the x-axis represents days from 0 to 27.](image-url)
### Comparison of rotifers production at three different stocking densities

<table>
<thead>
<tr>
<th>Stocking Density (ind.ml⁻¹)</th>
<th>Daily Production (rotifers.day⁻¹)</th>
<th>SGR (µ)</th>
<th>Σ Water Consumption (l)</th>
<th>Σ Food (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>2.2 × 10⁹ (45%)</td>
<td>0.6 ± 0.13</td>
<td>9760</td>
<td>21</td>
</tr>
<tr>
<td>5000</td>
<td>2.1 × 10⁹ (37%)</td>
<td>0.4 ± 0.07</td>
<td>7850</td>
<td>25</td>
</tr>
<tr>
<td>7000</td>
<td>1.7 × 10⁹ (21%)</td>
<td>0.3 ± 0.06</td>
<td>4500</td>
<td>28</td>
</tr>
</tbody>
</table>
## Bacterial count

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>MA</th>
<th>TCBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>water</td>
<td>$1.9 \times 10^5$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>rotifer</td>
<td>$3.5 \times 10^3$/rot.</td>
<td>$3.3 \times 10^2$/rot.</td>
</tr>
<tr>
<td>7</td>
<td>culture after protein skimmer</td>
<td>$3.4 \times 10^6$</td>
<td>$1.6 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>after biofilter</td>
<td>$1.8 \times 10^5$</td>
<td>$2.2 \times 10^4$</td>
</tr>
<tr>
<td>15</td>
<td>culture</td>
<td>$3.4 \times 10^6$</td>
<td>$3.8 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>after protein skimmer</td>
<td>$4.1 \times 10^5$</td>
<td>$3.5 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>after biofilter</td>
<td>$4.9 \times 10^5$</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>culture</td>
<td>$2.3 \times 10^5$</td>
<td>$3.0 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>after protein skimmer</td>
<td>$2.8 \times 10^5$</td>
<td>$5.5 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>after biofilter</td>
<td>$3.5 \times 10^4$</td>
<td>0</td>
</tr>
</tbody>
</table>
Changes in bacterial communities in rotifer cultures (DGGE)

recirculation technique

Week 1  Week 2  Week 3  Week 4

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Effect on rotifer quality

Rotifer size (µ)

- Week 1: 170 µ
- Week 2: 200 µ
- Week 3: 190 µ
- Week 4: 180 µ
✓ Effect on rotifer quality

Formulation of an experimental DHA recirculation diet

Nutritional content of rotifers (mg.g⁻¹)

- DHA : 10
- EPA : 5
- DHA/EPA : 2
- Σ(n-3) HUFA : 19
Cost estimation

**Batch System**
- Feed: 12.5%
- Labour: 14.8%
- Depreciation: 11%
- Others: 6.5%
- Investment cost: 55.2%
- Total cost: 94,396 €uro

**Recirculation System**
- Feed: 20.7%
- Labour: 9%
- Depreciation: 13.3%
- Others: 5%
- Investment cost: 52%
- Total cost: 54,000 €uro
Conclusions

• Reliable daily production of $2.0 \times 10^9$ rotifers harvested from the recirculation system during long term culture period (3 weeks)

• More efficient production obtained at 3000 ind./ml stocking density due to a better food conversion rate and higher daily rotifer production

• Stable microflora during the culture period with control on Vibrio’s

• Stable nutritional content of harvested rotifers, possibility to enrich rotifers with DHA during the rearing period no detrimental/oxydation effect of ozone

• Cost efficient production method