Effects of the applied organic loading rate on the selection of a PHA-storing biomass in a Sequencing Batch Reactor with uncoupled Carbon and Nitrogen feeding

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Polyhydroxyalkanoates (PHA)

**Product related Pro’s**
- Family of copolymers with tunable composition
- Main constituent of several bioplastics

- Biodegradable commodity film
- Packaging interlayer film
- Specialty durables (such as electronics)
- Slow C-release system for groundwater remediation

(Kunansudari, Exp Polym Let 2010)
Microbial mixed cultures process

Feedstock → Anaerobic Reactor → VFA → Aerobic Selection Reactor → PHA Production Reactor

Initial mixed consortium (activated sludge)

Enriched consortium (PHA-producing biomass)

VFA

End of the feast phase

feast-famine

O₂ (mg/L)

VFA - PHA (mgCOD/L)

t (min)
Aim of the study

Selection and enrichment of a PHA-producing biomass by applying an uncoupled C/N strategy

✓ Influence of the increasing applied OLR ➔
  4.25 gCOD/L d (Run A)
  8.50 gCOD/L d (Run B)
  12.75 gCOD/L d (Run C)

✓ Comparison with a previous study (Lorini et al., 2020)

✓ Exploring a higher OLR ➔
  18.0 gCOD/L d (Run D)

Selection of PHA-producing biomass (SBR)

Operative cycle (12 h)
- Feeding = 10 min
- Feast phase = 140 min
- Withdrawal = 3 min
- Nitrogen feeding = 5 min
- Famine phase = 562 min

Working volume = 1L

Organic load rate (OLR)
- 4.25; 8.5; 12.75; 18.0 gCOD/L d

VFA (85% Acetic acid; 15% Propionic acid)

T= 25°C
VFA, DO, PHA, NH$_4^+$ trends during a complete SBR cycle

Run B
OLR = 8.5 gCOD/L d

- Complete VFA depletion
- N-feeding

PHA and NH$_4^+$ consumption

Good selective pressure on the microbial consortium
Day of operation

End of the cycle
End of the feast

4.25 gCOD/Ld
8.5 gCOD/Ld
12.75 gCOD/Ld
18.0 gCOD/Ld
Comparison with the previous study

Dramatical decrease of the $Y_{P/S}$
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Run A</th>
<th>Run B</th>
<th>Run C</th>
<th>Run D</th>
<th>(Lorini et al. 2020)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLR (gCOD/L d)</td>
<td>4.25</td>
<td>8.5</td>
<td>12.75</td>
<td>18</td>
<td>4.25     8.5  12.75</td>
</tr>
<tr>
<td>Feast phase/cycle length ratio (h/h, %)</td>
<td>29.2 ± 2.9</td>
<td>28.7 ± 1.5</td>
<td>29.4 ± 1.6</td>
<td>39.4 ± 2.3</td>
<td>21.0 ± 0.6</td>
</tr>
<tr>
<td>PHA concentration (end of cycle; mg/L)</td>
<td>76 ± 8</td>
<td>505 ± 40</td>
<td>1076 ± 121</td>
<td>1168 ± 256</td>
<td>235 ± 23</td>
</tr>
<tr>
<td>PHA concentration (end of feast; mg/L)</td>
<td>601 ± 50</td>
<td>1780 ± 80</td>
<td>3080 ± 121</td>
<td>2049 ± 110</td>
<td>807 ± 58</td>
</tr>
<tr>
<td>PHA content (end of feast; gPHA/gVSS)</td>
<td>0.34 ± 0.03</td>
<td>0.52 ± 0.03</td>
<td>0.62 ± 0.02</td>
<td>0.36 ± 0.05</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>Storage Yield (YP/S feast; COD/COD)</td>
<td>0.42 ± 0.04</td>
<td>0.58 ± 0.03</td>
<td>0.64 ± 0.05</td>
<td>0.10 ± 0.06</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>HV content (end of feast; gHV/gPHA)</td>
<td>0.15 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Nitrogen concentration (end of the cycle; mgN/L)</td>
<td>20 ± 3</td>
<td>34 ± 3</td>
<td>10 ± 3</td>
<td>101 ± 14</td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

Conclusions and future perspectives

- At OLR ranging between 4.25 - 12.75 g COD/L d, the feast/famine regime was easy established and a strong PHA-storing biomass selection was observed in line with the high storage yield.

- At very high OLR (18 g COD/L d) the system was unstable and the storing capacity of the microbial community was strongly affected.

- The high PHA content achieved may allow simplifying the process by skipping the traditional accumulation step.

- The exploitation of nutrient deficient organic waste (paper mill and olive oil mill wastewaters, cheese whey permeate or sugar-cane molasses) may be realized including a nitrogen and phosphorus addition in the famine phase.
Thanks for your attention