Polyhydroxyalkanoate production from yeast industry wastewater using mixed microbial culture

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The Project steps are:

1. PHA production in activated sludge with using yeast industry wastewater (YWW) as a feeding stream,
2. PHA extraction with using different extraction methods,
3. Nanofiber production

**Solvent extraction**
**Supercritical carbon dioxide**
**Hydrodynamic cavitation**

**Waste to Biopolymer**

**Electrospinning**
**Nanofiber characterization**

**Enrichment**
**Activated sludge**

**Accumulation**

**Extraction**

**Characterization**
I. Introduction to PHB

II. PHB produced microorganisms and substrates

III. Experimental Procedure

IV. Results

V. Conclusion and Future Work
I. Introduction to PHB

- PHB was the first PHA to be identified in 1926 by in the bacterium Bacillus megaterium.

- PHB is the most widely studied and best-characterized member of PHAs.

n=1, R=metil => poli-3-hidroksibütirat (PHB)
### II. PHB produced microorganisms and substrates

<table>
<thead>
<tr>
<th>Food waste source</th>
<th>Microorganisms(s)</th>
<th>PHA polymer type</th>
<th>Cultivation</th>
<th>Dry cell weight (g l⁻¹)</th>
<th>Maximum PHA production reported (g PHA g⁻¹ dcw)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent coffee grounds oil</td>
<td>Cupriavidus necator DSM 428</td>
<td>PHB</td>
<td>Fermenter, fed-batch</td>
<td>16.7</td>
<td>78.40%</td>
<td>Cruz et al. (2014)</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Wheat bran</td>
<td>PHBV</td>
<td>Continuous feeding</td>
<td>22.7</td>
<td>72.60%</td>
<td>Du and Yu (2002)</td>
</tr>
<tr>
<td>Starch</td>
<td>Azotobacter chroococcum</td>
<td>PHB</td>
<td>Fermenter, batch</td>
<td>54</td>
<td>46%</td>
<td>Kim (2000)</td>
</tr>
<tr>
<td>Sugarcane molasses</td>
<td>Bacillus megaterium</td>
<td>PHB</td>
<td>Fermenter, fed-batch</td>
<td>72.2</td>
<td>42%</td>
<td>Kulpreecha et al.</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Bacillus firmus NII</td>
<td>PHB</td>
<td>Fermenter, batch</td>
<td>1.9</td>
<td>89%</td>
<td>Sindhu et al. (2013)</td>
</tr>
<tr>
<td>Molasses</td>
<td>Psuedomonas</td>
<td>PHA</td>
<td>Flask, batch</td>
<td>10.54</td>
<td>20.63%</td>
<td>Chaudhry et al. (2014)</td>
</tr>
</tbody>
</table>

**Table 3: Production of PHAs from anaerobically digested food waste.**

<table>
<thead>
<tr>
<th>Food scraps from cafeteria</th>
<th>C. necator</th>
<th>PHBV</th>
<th>Fermenter, batch</th>
<th>22.7</th>
<th>72.60%</th>
<th>Du and Yu (2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitchen waste</td>
<td>C. necator</td>
<td>PHB</td>
<td>Fermenter, batch</td>
<td>4.6</td>
<td>52.79%</td>
<td>Omar et al. (2011)</td>
</tr>
<tr>
<td>Fermented molasses</td>
<td>Mixed microbial culture</td>
<td>PHBV</td>
<td>Fermenter, pulse feed</td>
<td>56%</td>
<td></td>
<td>Albuquerque et al. (2011)</td>
</tr>
<tr>
<td>Olive oil mill pomace</td>
<td>Activated sludge consortia</td>
<td>PHBV</td>
<td>SBR</td>
<td>39%</td>
<td></td>
<td>Waller et al. (2012)</td>
</tr>
</tbody>
</table>

III. Experimental Procedure

**Parameter** | **Value**
--- | ---
pH | 7.5-7.88
COD (mg/L) | 2500-3430
ΣN (mg/L) | 100-200
ΣP (mg/L) | 6.7-21.9
Suspended solids (SS) (mg/L) | 450-500
Total volatile fatty acids (VFA) (mg/L) | 950-3000

- **Acetic acid**: %90
- **Propionic acid**: %3
- **Butyric acid**: %3
- **Isobutyric acid**: %2
- **i-valeric acid**: %1
- **Valeric acid**: %1
III. Experimental Procedure

**Inoculum:** activated sludge

**Feeding stream:** anaerobically pre-treated yeast industry wastewater (YWW)

Two steps PHB production from yeast industry wastewater in activated sludge was investigated:

1) feeding of activated sludge with anaerobically pre-treated yeast industry wastewater (enrichment reactor)

2) PHB accumulation by pulse addition in the excess sludge of enrichment reactor.
III. Experimental Procedure

**Analytical methods:**
- Chemical oxygen demand (COD),
- Total suspended solids (TSS),
- Volatile suspended solids (VSS),
- PHB content (%cell dry weight (CDW))
- Dissolved oxygen (DO),
- PHB extraction
IV. RESULTS

- COD value of wastewater was increased step by step to increase the TSS.

- End of the 20 days operation while SBR still continues for the enrichment stage, excess sludge was used for the accumulation stage.
IV. RESULTS

The wastewater was fed by pulse addition (4 pulses) controlled by the DO concentration.

PHB storage was increased to 0.31 mg PHB/mg CDW at the end of 4 pulses which was 0.14 mg PHB/mg CDW at the beginning of the accumulation.
IV. RESULTS

FTIR and TGA analysis of biopolymer comparison with commercial PHB sample
VI. CONCLUSION and FUTURE WORK

• There are few study which are feeded to enrichment reactor with wastewater stream instead of synthetic acetate and mineral solution. In this study, the culture was able to accumulate 30% PHB (for CDW).

• Study is still going on with the accumulation experiments to increase the PHB content in the (MMC).

• Extraction experiments continue in parallel with the accumulation.

• Extracted polymers are going to be used for the nanofiber production

• End of this project, we will have developed new methods for the production of biopolymer by evaluating waste and extracting this polymer using environmentally friendly methods.
Thank you for your attention!

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