BIOREFINING OF SUGAR-BEET PROCESSING WASTES BY ANAEROBIC BIOTECHNOLOGY: Waste Stabilization and Bio-product Formation

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INTRODUCTION
“A major step for the development of a sustainable, industrial society will be the shift from our dependence on petroleum to the use of renewable resources” (Sauer et al. 2008)

Even though there are many efforts of producing renewable energy and resources from virgin raw materials, use of wastes as raw materials for renewable energy and resources is rather new.
There exist significant opportunities for value-added product formation from by-products/wastes through biotechnological approaches.

Recently, sugar-beet processing plants take measures to reduce energy consumption, recycle materials and energy and optimize the operation of manufacturing process in order to achieve waste minimization and sustainable production.

Parallel to the advances in the process control and environmental biotechnology, now anaerobic digestion of sugar-beet processing wastes, has the potential for achieving dual-goal of waste management and value-added product generation.
The main objective of this study was to investigate two of the possible exploitation routes of anaerobic digestion (acid-phase and methane-phase) for the treatment of sugar-beet processing wastes, while producing bioenergy and biobased products.

In the literature, most of the studies regarding the anaerobic digestion of sugar-beet processing wastes targeted to the biomethanation and waste stabilization of each individual waste stream, resulted from different processing lines.

However, wastewater, as the primary source of sugar industry related environmental problems, and beet-pulp, a by-product generated in vast amounts, can be managed in an integrated manner.

Although anaerobic treatment of wastewater is rather established, bioprocessing of beet-pulp is developing with a significant potential as alternative to conventional animal feeding practices.
Aim and Scope of the Study (2/2)

Not included in this presentation
Biomass has been considered as one of the main alternatives to fossil resources, being a renewable source for value-added product (biofuels, biochemicals etc.) formation in the last couple decades.

With the advances in the biotechnology, biofuels as well as a variety of chemicals such as alcohols, ketones, and organic acids, as well as biofuels like biodiesel, ethanol, methane and hydrogen can be manufactured by biological transformations.

This approach is known as “biorefining”.
Biorefineries Turn Biomass into Multiple Products

**Plant Science**
- Genomics
- Enzymes
- Metabolism
- Composition

**Production**
- Trees
- Grasses
- Agricultural Crops
- Agricultural Residues
- Animal Wastes
- Municipal Solid Waste

**Processing**
- Acid/Enzymatic hydrolysis
- Fermentation
- Bioconversion
- Chemical Conversion
- Gasification
- Combustion
- Co-firing

**End-Uses**
- Chemicals
- Plastics
- Functional Monomers
- Solvents
- Chemical Intermediates
- Phenolics
- Adhesives
- Hydraulic Fluids
- Paints
- Dyes, Pigments, and Ink
- Detergents
- Paper
- Fiber boards
- Solvents
- Adhesives
- Plastic filler
- Abrasives
- Transportation Fuels
- Electric Power
Feedstocks for Biorefining

Starch, Sugar & Oil
- Corn
- Wheat
- Soybean
- Barley
- Palm
- Triticale
- Sugar cane
- Canola

Cellulose / Hemicellulose
- Hybrid Poplar
- Grasses
- Forest residue
- Hemp
- Sawdust
- Cereal straw
- Yard waste

Livestock and Food Processing Wastes
- Manure
- Animal fats & used frying oils
- Cheese whey
- Food processing wastes
- Corn syrup
- Fruit processing wastes
- Milling byproducts
- Biorefinery wastes
How Much Biomass Could Be Produced?

Total potential in U.S. is in excess of 1 billion tons (about 21 Quads)

- Could supply 21% of U.S. energy demand, or
- 33% of U.S. transportation fuel

Anaerobic digestion is a biological process in which a group of microorganisms biodegrade the organic matter (substrate) in the absence of free molecular oxygen (O2).

As a result of this complex biological process, organic matter is mainly converted into a mixture of methane (CH4) and carbon dioxide (CO2) as well as new bacterial cells.

The biochemistry of anaerobic digestion involves stepwise reactions in four major stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. In each of these stages, different groups of bacteria are responsible for the bioconversion of intermediate metabolites into substrates for subsequent stages.
Volatile Fatty Acids (VFAs)

- VFAs are short-chain fatty acids such as acetic, propionic etc. acids.

- Being valuable chemical products, VFAs have diverse uses in the market. They are utilized for the manufacture of various organic compounds including alcohols, aldehydes, ketones, esters and olefins.

- As it is the case for most of other commodity chemicals, they are usually manufactured from fossil fuels through chemical synthesis. However fermentation, using renewable resources, is more preferable from the viewpoint of sustainable development and human health.

- There are various techniques, applied for the recovery of organic acids from fermentation broths, including; electrodialysis, ion-exchange, adsorption and liquid-liquid extraction.
Market Analysis / Demand

<table>
<thead>
<tr>
<th>Product</th>
<th>Demand 2023</th>
<th>Growth Outlook</th>
<th>Price Range 2005-2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>5.1 M lbs</td>
<td>2% per year through 2023, high 60.27 lb</td>
<td>Current: Low $5.12/lb, High $53.32/lb</td>
</tr>
<tr>
<td>Propionic Acid</td>
<td>5.1 M lbs</td>
<td>3.5% per year through 2023, high 66.44 lb</td>
<td>Current: Low $4.59/lb, High $50.50/lb</td>
</tr>
</tbody>
</table>

Market Demands for Products Years 2005-2025

- Acetic Acid
- Propionic Acid
WASTE CHARACTERISTICS
# Waste Characteristics

## Table 3.1. Wastewater characteristics (1 hr settled)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tCOD</td>
<td>6621 ± 113.2</td>
</tr>
<tr>
<td>sCOD</td>
<td>6165 ± 517.1</td>
</tr>
<tr>
<td>TS</td>
<td>6062 ± 53.0</td>
</tr>
<tr>
<td>VS</td>
<td>2832 ± 25</td>
</tr>
<tr>
<td>TSS</td>
<td>665 ± 21.2</td>
</tr>
<tr>
<td>VSS</td>
<td>335 ± 7.1</td>
</tr>
<tr>
<td>pH</td>
<td>6.82</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>1760</td>
</tr>
<tr>
<td>TKN</td>
<td>10</td>
</tr>
<tr>
<td>P&lt;sub&gt;Total&lt;/sub&gt;</td>
<td>2.7</td>
</tr>
<tr>
<td>tVFA (as H-Ac)</td>
<td>1115 ± 20</td>
</tr>
<tr>
<td>H-Ac</td>
<td>394 ± 5</td>
</tr>
<tr>
<td>H-Pr</td>
<td>610 ± 12</td>
</tr>
<tr>
<td>H-Bu</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>Calcium (Ca²⁺)</td>
<td>378 ± 5.7</td>
</tr>
</tbody>
</table>

## Table 3.2. Pressed beet-pulp characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>85 ± 0.1</td>
</tr>
<tr>
<td>TS (%)</td>
<td>15 ± 0.1</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>94 ± 0.01</td>
</tr>
<tr>
<td>COD (g/g dry weight)</td>
<td>1.22 ± 0.15</td>
</tr>
<tr>
<td>TKN (%TS)</td>
<td>7.28</td>
</tr>
<tr>
<td>P&lt;sub&gt;Total&lt;/sub&gt; (%TS)</td>
<td>1.0 ± 0.28</td>
</tr>
</tbody>
</table>
EXPERIMENTAL STUDIES
Set-up 1: BMP Assay
Set-up 1: BMP Assay

- Separate and co-digestion of wastewater and beet-pulp were studied in 250-ml reactors with effective volume of 150 ml.

- Reactors were incubated in a temperature controlled room at 35 ± 1 °C.

- Continuous mixing was applied at 175 rpm for 38 days of operation.

- Experimental set-up was designed to investigate the effect of separate and co-digestion of wastewater and the beet-pulp.

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### Operational Scheme

**Table 3.3: Initial conditions inside the reactors**

<table>
<thead>
<tr>
<th>Reactor</th>
<th>pH*</th>
<th>F/M (g COD/g VSS)</th>
<th>Wastewater COD (mg/L)</th>
<th>Beet-pulp COD (mg/L)</th>
<th>Total COD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>8.13</td>
<td>0.51</td>
<td>4500</td>
<td>-</td>
<td>4500</td>
</tr>
<tr>
<td>R2</td>
<td>7.59</td>
<td>0.26</td>
<td>-</td>
<td>2250</td>
<td>2250</td>
</tr>
<tr>
<td>R3</td>
<td>8.01</td>
<td>0.51</td>
<td>-</td>
<td>4500</td>
<td>4500</td>
</tr>
<tr>
<td>R4</td>
<td>7.96</td>
<td>1.02</td>
<td>-</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>R5</td>
<td>7.97</td>
<td>2.05</td>
<td>-</td>
<td>18000</td>
<td>18000</td>
</tr>
<tr>
<td>R6</td>
<td>7.88</td>
<td>0.77</td>
<td>4500</td>
<td>2250</td>
<td>6750</td>
</tr>
<tr>
<td>R7</td>
<td>7.70</td>
<td>1.02</td>
<td>4500</td>
<td>4500</td>
<td>9000</td>
</tr>
<tr>
<td>R8</td>
<td>7.71</td>
<td>1.54</td>
<td>4500</td>
<td>9000</td>
<td>13500</td>
</tr>
<tr>
<td>R9</td>
<td>7.67</td>
<td>2.56</td>
<td>4500</td>
<td>18000</td>
<td>22500</td>
</tr>
<tr>
<td>Control</td>
<td>7.48</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Prior to purging with 75% N₂ + 25% CO₂ gas mixture.
Set-up 1: BMP Assay

- During the first 10 days of operation, reactors produced 57.0–85.1% of their total biogas productions (as of day 38), without any indication of a significant inhibition.

- It is clear from the figure that, total biogas production was proportional to initial feed concentration.

- Accordingly, highest biogas production was calculated as 1725 mL in R9 which had an initial COD concentration of 22500 mg/L.
Set-up 1: BMP Assay

- Results point that the highest values of methane yield (321.6 mL/g COD added) COD removal (87.3%) and VS reduction (89.3%) were observed at R1, which was fed only by wastewater.

- However, the treatment efficiencies obtained (63.7–87.3% COD removal and 69.6–89.3% VS reduction) are indications of high biodegradability for both wastewater and beet-pulp for all F/M values investigated.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Initial F/M (g COD/g VSS)</th>
<th>Final pH</th>
<th>COD removal (%)</th>
<th>VS reduction (%)</th>
<th>CH₄ yield (mL/g COD added)</th>
<th>% of total biogas (produced in first 10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>7.53</td>
<td>-</td>
<td>28.4</td>
<td>-</td>
<td>29.3</td>
</tr>
<tr>
<td>R1</td>
<td>0.51</td>
<td>7.58</td>
<td>87.3</td>
<td>89.3</td>
<td>321.6</td>
<td>85.1</td>
</tr>
<tr>
<td>R2</td>
<td>0.26</td>
<td>7.43</td>
<td>79.6</td>
<td>82.2</td>
<td>296.4</td>
<td>64.8</td>
</tr>
<tr>
<td>R3</td>
<td>0.51</td>
<td>7.48</td>
<td>79.8</td>
<td>82.5</td>
<td>261.8</td>
<td>57.0</td>
</tr>
<tr>
<td>R4</td>
<td>1.02</td>
<td>7.46</td>
<td>63.7</td>
<td>69.6</td>
<td>238.6</td>
<td>68.1</td>
</tr>
<tr>
<td>R5</td>
<td>2.05</td>
<td>7.45</td>
<td>66.6</td>
<td>73.1</td>
<td>226.7</td>
<td>66.4</td>
</tr>
<tr>
<td>R6</td>
<td>0.77</td>
<td>7.48</td>
<td>84.2</td>
<td>84.4</td>
<td>311.9</td>
<td>83.8</td>
</tr>
<tr>
<td>R7</td>
<td>1.02</td>
<td>7.47</td>
<td>81.5</td>
<td>80.2</td>
<td>299.9</td>
<td>84.2</td>
</tr>
<tr>
<td>R8</td>
<td>1.54</td>
<td>7.47</td>
<td>72.3</td>
<td>73.0</td>
<td>276.9</td>
<td>83.6</td>
</tr>
<tr>
<td>R9</td>
<td>2.56</td>
<td>7.48</td>
<td>64.1</td>
<td>75.1</td>
<td>235.8</td>
<td>75.2</td>
</tr>
</tbody>
</table>
Set-up 1: BMP Assay

- Since, hydrolysis is the overall rate controlling step in anaerobic digestion of beet-pulp, in this study methane production data was analyzed by first-order kinetics:

\[ G_t = G_f \left(1 - e^{-kt}\right) \]

Where:
- \( G_t \): Cumulative methane generation at time \( t \) (mL),
- \( G_f \): Ultimate methane generation (mL),
- \( k \): First-order rate constant (day\(^{-1}\))
- \( t \): Time (days).
Set-up 1: BMP Assay

- There was a remarkable difference, in terms of $k$ values, between wastewater added (0.081–0.143 day⁻¹) and non-added reactors (0.028–0.050 day⁻¹).

- Waste water addition increased methane production rate, rather than increasing the ultimate biodegradability.

- Wastewater, containing easily degradable carbohydrates, could be consumed by the anaerobic bacteria without requiring a lag phase for hydrolysis. Meanwhile the hydrolysis of the beet pulp was underway.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>$R^2$</th>
<th>Produced total CH$_4$ (mL)</th>
<th>$G_f$ (mL)</th>
<th>$k$ (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9687</td>
<td>71.1</td>
<td>128.0 ± 41.3</td>
<td>0.025 ± 0.011</td>
</tr>
<tr>
<td>R1</td>
<td>0.9854</td>
<td>217.1</td>
<td>214.3 ± 6.9</td>
<td>0.143 ± 0.016</td>
</tr>
<tr>
<td>R2</td>
<td>0.9702</td>
<td>100.2</td>
<td>139.7 ± 27.4</td>
<td>0.037 ± 0.012</td>
</tr>
<tr>
<td>R3</td>
<td>0.9392</td>
<td>176.7</td>
<td>293.8 ± 120.5</td>
<td>0.028 ± 0.017</td>
</tr>
<tr>
<td>R4</td>
<td>0.9196</td>
<td>322.1</td>
<td>410.6 ± 97.8</td>
<td>0.050 ± 0.022</td>
</tr>
<tr>
<td>R5</td>
<td>0.9184</td>
<td>612.0</td>
<td>818.7 ± 24.1</td>
<td>0.045 ± 0.022</td>
</tr>
<tr>
<td>R6</td>
<td>0.9829</td>
<td>316.2</td>
<td>316.0 ± 11.6</td>
<td>0.135 ± 0.016</td>
</tr>
<tr>
<td>R7</td>
<td>0.9845</td>
<td>404.8</td>
<td>409.3 ± 14.8</td>
<td>0.129 ± 0.015</td>
</tr>
<tr>
<td>R8</td>
<td>0.9816</td>
<td>560.7</td>
<td>578.4 ± 25.5</td>
<td>0.113 ± 0.015</td>
</tr>
<tr>
<td>R9</td>
<td>0.9685</td>
<td>795.8</td>
<td>872.2 ± 70.9</td>
<td>0.081 ± 0.016</td>
</tr>
</tbody>
</table>
Set-up 2: Optimization of Anaerobic Acidification
Set-up 2: Optimization of Anaerobic Acidification

- 250 mL reactors with effective volume of 150 mL were operated by daily fed-batch feeding strategy as continuously mixed acidogenic reactors.

- Reactors were kept continuously mixing at 175 rpm in a temperature controlled room (35 ± 1 °C) for 41 days.

- First 20 days of operation was carried out by adding external alkalinity. Then, the reactors were operated 21 more days without adding any external alkalinity.

- Different HRTs, mixing ratios and OLRs were used.

### Table 3.4. Operational parameters of the reactors

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Feed</th>
<th>HRT (Days)</th>
<th>Waste mixing ratio (WW:P)</th>
<th>OLR (g COD/L-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Stock #1</td>
<td>2</td>
<td>1:0</td>
<td>2.7</td>
</tr>
<tr>
<td>R2</td>
<td>Stock #2</td>
<td>2</td>
<td>1:0.5</td>
<td>4</td>
</tr>
<tr>
<td>R3</td>
<td>Stock #3</td>
<td>2</td>
<td>1:1</td>
<td>5.4</td>
</tr>
<tr>
<td>R4</td>
<td>Stock #1</td>
<td>4</td>
<td>1:0</td>
<td>1.35</td>
</tr>
<tr>
<td>R5</td>
<td>Stock #2</td>
<td>4</td>
<td>1:0.5</td>
<td>2</td>
</tr>
<tr>
<td>R6</td>
<td>Stock #3</td>
<td>4</td>
<td>1:1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

### Table 3.5. Compositions of stock solutions

<table>
<thead>
<tr>
<th>Composition</th>
<th>Stock #1</th>
<th>Stock #2</th>
<th>Stock #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater (mg/L as tCOD)</td>
<td>5300</td>
<td>5300</td>
<td>5300</td>
</tr>
<tr>
<td>Pulp (mg/L as tCOD)</td>
<td>–</td>
<td>2650</td>
<td>5300</td>
</tr>
<tr>
<td>tCOD (mg/L)</td>
<td>5300</td>
<td>7950</td>
<td>10600</td>
</tr>
<tr>
<td>sCOD (mg/L)</td>
<td>4932 ± 72</td>
<td>5211 ± 421</td>
<td>5318 ± 288</td>
</tr>
<tr>
<td>pH</td>
<td>8.02</td>
<td>7.99</td>
<td>8.08</td>
</tr>
</tbody>
</table>
Set-up 2: Optimization of Anaerobic Acidification

- VFA productions were initiated from the beginning of the reactor operation which resulted in the expected pH drops.

- External alkalinity addition kept the pH range between 6.8-7.6 during the first 20 days of operation.

- Since the pH range of 4–6.5 is accepted as optimum for the growth of anaerobic acidogenic microorganisms, reactors were operated 21 more days with no alkalinity addition. This resulted in a pH range of 5.5-7.5.
Set-up 2: Optimization of Anaerobic Acidification

Volatile Fatty Acid Production

- 2 days of HRT yielded high tVFA concentrations (2159–3635 mg/L as H-Ac), which is the indication of high acidogenic activity and lower methanogenic activity.

- In all of the reactors, main acidification products were H-Ac (40.3–49.2% w/w of tVFA), H-Pr (36.3–42.6% w/w of tVFA) and H-Bu (3.6–7.5% w/w of tVFA) comprising 88.3–96.2% of tVFAs.
Set-up 2: Optimization of Anaerobic Acidification

- After cessation of alkalinity addition, methane percentages of the reactors were slightly decreased following the decreases in pH values.

- The lowest methane percentage (5.6%) was observed at R3 in the period without alkalinity addition.

- This result indicates that, the optimum conditions for inhibition of methanogenic activity were 2 days of HRT and 5.5–6.0 range of pH.

### Table 4.3: Biogas compositions, recorded at corresponding steady-state period

<table>
<thead>
<tr>
<th>Reactor</th>
<th>External Alkalinity</th>
<th></th>
<th>No External Alkalinity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH₄ (%)</td>
<td>CO₂ (%)</td>
<td>CH₄ (%)</td>
<td>CO₂ (%)</td>
</tr>
<tr>
<td>R1</td>
<td>25.3 ± 1.7</td>
<td>74.7 ± 1.7</td>
<td>23.2 ± 5.9</td>
<td>76.8 ± 5.9</td>
</tr>
<tr>
<td>R2</td>
<td>16.3 ± 2.1</td>
<td>83.7 ± 2.1</td>
<td>16.7 ± 3.3</td>
<td>83.3 ± 3.3</td>
</tr>
<tr>
<td>R3</td>
<td>6.4 ± 1.5</td>
<td>93.7 ± 1.5</td>
<td>5.6 ± 0.5</td>
<td>94.4 ± 0.5</td>
</tr>
<tr>
<td>R4</td>
<td>53.4 ± 0.4</td>
<td>46.6 ± 0.4</td>
<td>49.8 ± 2.1</td>
<td>50.2 ± 2.1</td>
</tr>
<tr>
<td>R5</td>
<td>51.6 ± 3.3</td>
<td>48.4 ± 3.3</td>
<td>45.1 ± 2.6</td>
<td>54.9 ± 2.6</td>
</tr>
<tr>
<td>R6</td>
<td>44.5 ± 2.7</td>
<td>55.5 ± 2.7</td>
<td>39.5 ± 2.2</td>
<td>60.5 ± 2.2</td>
</tr>
</tbody>
</table>
The highest acidification degrees (60.3–64.2%) were observed in R1, which was only fed by wastewater and operated at 2 days of HRT.

Operational conditions of R3 lead to the highest tVFA concentrations (3635 ± 209 mg/L as H-Ac) with an acidification degree of 46.9% at the highest OLR of 5.4 g COD/L-d.
Set-up 3:
Recovery of VFAs
by
Liquid-Liquid Extraction
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

- Simultaneous acidification of wastewater and beet-pulp were achieved in an anaerobic reactor composed of a 550 mL reactor with an effective volume of 500 mL.

- Based on the results from Set-up 2, reactors were operated at 2-day HRT and 5.4 g COD/L-day OLR.

- During the course of the operation, pH decreased while sCOD and VFA concentrations increased. This indicated simultaneous hydrolysis and acidification of organic matter.
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

**Acidification Performance of the Reactor**

- **Significant removal of SS** (37.5%) and **VSS** (40.5%) were observed through the hydrolysis of particulate matter.

- **Main acidification products** were **acetic** (48.4% w/w of tVFA), **propionic** (38.1%), **n-butyric** (5.9%) and **n-valeric** (3.2%) acids, corresponding 95.6% of tVFA.

- **Effluents** of five consecutive days (day 16–20) were collected together in a flask and kept at +4 °C, to be used further in extraction experiments.

---

Table 4.4. Influent and effluent characteristics of acidification reactor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent (mg/L)</th>
<th>Effluent (mg/L)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tCOD</td>
<td>10600</td>
<td>9708 ± 555</td>
<td>-8.4</td>
</tr>
<tr>
<td>sCOD</td>
<td>5318 ± 288</td>
<td>5755 ± 30</td>
<td>+8.2</td>
</tr>
<tr>
<td>TS</td>
<td>9193</td>
<td>9041 ± 189</td>
<td>-1.7</td>
</tr>
<tr>
<td>VS</td>
<td>6348</td>
<td>5495 ± 389</td>
<td>-13.4</td>
</tr>
<tr>
<td>SS</td>
<td>4832</td>
<td>3020 ± 57</td>
<td>-37.5</td>
</tr>
<tr>
<td>VSS</td>
<td>4268</td>
<td>2540 ± 85</td>
<td>-40.5</td>
</tr>
<tr>
<td>tVFA (as H-Ac)</td>
<td>892 ± 16</td>
<td>2913 ± 152</td>
<td>+37.8*</td>
</tr>
<tr>
<td>pH</td>
<td>7.65</td>
<td>5.5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Degree of acidification, computed as described in Part 4.2.2.*
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

Experimental Set-up and Procedure

Solvents were prepared at 30 °C by dissolving TOPO (trioctylphosphine oxide) in kerosene with different ratios (5, 10, and 20% wt). Then the prepared solvents were used for the extraction in two different pH conditions (2.5 and 5.5). In the first run, prior to extraction, pH of the aqueous phase was decreased to 2.5, which is smaller than pKₐ values of VFAs (pKₐ = 4.8) to ensure the predominance of undissociated forms (i.e. acid forms) of VFAs. Then, in the second run, the aqueous phase (filtered fermentation broth) was subjected to extraction without any pH adjustments (at pH 5.5) to observe the effect of pH.

For the extraction of VFAs, 10 mL of each aqueous and organic phase were mixed in a 30 mL separatory funnel, shaking at room temperature for 5 minutes. In this equilibrium condition, the extraction mixture was allowed to settle for 1–3 minutes. After two phases were separated by gravity, aqueous phase was subjected to VFA and COD analyses. Then, organic phase VFA concentrations ([HA]_{org}) were determined by mass balance.
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

Percent Recovery

The percent weight of acid, transferred from the aqueous phase into organic phase was expressed as the percent recovery of corresponding acid.

So as to compare total extraction efficiencies of different runs, all VFAs were expressed as acetic acid concentration (mg L\(^{-1}\) as H-Ac) and summation of these concentrations were expressed as total VFA concentration (tVFA) as well as separate VFAs (acetic, propionic, n-butyric, and n-valeric) for recovery calculations.

\[
\% \text{ Recovery} = \frac{\left( [HA]_{org} \times V_{org} \right)_{eq}}{\left( [HA]_{aq} \times V_{aq} \right)_{eq}}
\]

Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

Extraction at pH 2.5

- At pH 2.5, percent recoveries of VFAs were varied between 43.3 and 97.6%, depending on the type of the acid extracted and the concentration of TOPO in kerosene.

- The highest recovery percentages (94.2–97.6%) were observed for valeric acid at pH 2.5.

- Still, considerable degree of recovery (43.3–60.6%) was observed for acetic acid.
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

Extraction at pH 5.5

- The effect of TOPO concentration in kerosene was not very significant on extraction efficiency both at pH 2.5 and 5.0.

- At pH 5.0, the recovery percentages of acetic and propionic acids, which are two major contributors to tVFA concentration, are remained as low as 29.3–30.1%.
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

- The recovery of VFAs, which contribute to the majority of sCOD in acidified waste streams, can also be considered as a means of wastewater pre-treatment.

- Up to 71.8% COD removals were achieved, at 20% TOPO in kerosene at pH 2.5, while the removal efficiencies remained between 19.1–22.3% at pH 5.5.

- Higher chain-length VFAs are more susceptible to extraction. This tendency can lead to promising results in terms of waste stabilization, since their contribution to COD is higher than shorter chain-length VFAs (Table 4.9).

Table 4.9. Theoretical COD equivalence of VFAs

<table>
<thead>
<tr>
<th>VFA</th>
<th>COD* equivalent (g/g Acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>1.066</td>
</tr>
<tr>
<td>Propionic</td>
<td>1.512</td>
</tr>
<tr>
<td>n-Butyric</td>
<td>1.816</td>
</tr>
<tr>
<td>n-Valeric</td>
<td>2.036</td>
</tr>
</tbody>
</table>

*(Yilmaz and Demirer 2008).*
CONCLUSIONS
Set-up 1: BMP Assay

- The observed treatment efficiencies *(63.7–87.3% COD removal and 69.6–89.3% VS reduction)* were indications of high biodegradability for both wastewater and beet-pulp.

- Anaerobic co-digestion of wastewater and beet-pulp is promising since wastewater addition significantly increases the rate of biomethanation of beet-pulp.
Set-up 2: Optimization of Anaerobic Acidification

- Sugar industry wastewater and beet-pulp can **simultaneously** be converted to VFAs in acidogenic anaerobic reactors with **considerable acidification degrees** (43.8–52.9%).

- Optimum operational conditions for anaerobic acidification were selected as **2-day HRT and 1:1 waste mixing ratio (in terms of COD)** without external alkalinity addition.
Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

- pH 2.5 was determined as optimum. At this pH, percent recoveries of VFAs were observed between 43.3 and 97.6%.

- At pH 2.5, up to 71.8% COD removals were achieved. While the removal efficiencies remained between 19.1–22.3% at pH 5.5.
In order to establish an integrated approach for the management of sugar-beet processing wastes by anaerobic digestion, further research is needed in:

i. Evaluation of the effect of operational parameters on anaerobic acid-phase and methane-phase digestion like, nutrient availability (anaerobic digestion without BM) and physical pretreatment of beet-pulp,

ii. Developing a basis for the comparison of the anaerobic acid-phase and methane-phase digestion routes by means of a economical and technical feasibility study

iii. Investigating the applicability in pilot-scale and/or demonstration studies on-site, and
I would like to acknowledge my co-workers:

✍️ Mr. Emrah Alkaya
✍️ Mr. Serkan Kaptan
✍️ Miss. Leyla Ozkan
✍️ Dr. Sibel Uludag-Demirer

Thank you for your attention.

If you have questions or ideas to discuss, please do not hesitate to contact:

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