Biodiesel production from *Acrocomia aculeata* acid oil by (enzyme/enzyme) hydroesterification process: Use of vegetable lipase and fermented solid as low-cost biocatalysts

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Monoalkyl esters produced from vegetable oils or animal fats that has similar properties to petro-diesel and its burning results in lower emissions of particulates, CO, SOx and aromatic hydrocarbons.

**Brazil** → The National Program for Use and Production of Biodiesel (PNPB) of 2005 established a minimum percentage of biodiesel blended with petro-diesel (2%, named B2) in January 2008, which has been increasing over time. Nowadays, Brazil is using B6.

Brazil is the fourth world producer of biodiesel with a monthly production higher than 200,000 m³ per month.

Source: ANP – Jun/14
INTRODUCTION

CATALYSTS

CURRENT INDUSTRIAL PROCESS

- Difficulty of separating the catalyst from the glycerol;
- Production of highly alkaline wastewater;
- Requirement of high-quality raw materials with low contents of free fatty acids (FFAs) and water in order to avoid soap formation (high cost of raw materials).

LIPASES

- High selectivity;
- Mild operating conditions;
- High purity of the generated products (glycerol and esters);
- Use of oils with high acidity (cheaper).

Alternative vegetable non-edible oil crops → Acid oil from macauba (Acrocomia aculeata) pulp

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**INTRODUCTION**

**MACAUBA**

- Macauba is a native oleaginous palm tree of the Brazilian Cerrado;
- Candidate crop that can be used as an alternative feedstock to regional industries in Brazil;
- High productivity → potencial to produce 4 t of oil/ha;
- The oil is rich in oleic acid → generates a high quality biodiesel with high content of monounsaturated esters;
- Oil has high acidity and low market value → cannot be used as feedstock for biodiesel production by conventional alkaline route.

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INTRODUCTION

ENZYME/ENZYME HYDROESTERIFICATION ROUTE

Hydrolysis of all glycerides (mono-, di- and triglycerides) that produces FFAs and glycerol.

Esterification of the FFAs with a short chain alcohol to obtain esters (biodiesel) and water.

Vegetable enzyme (VE)

Microbial lipase (obtained by Solid State Fermentation-SSF)

New approach to lower the enzyme costs and consequently make the biodiesel obtained by enzymatic route more cost competitive!

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The aim of this study was to produce biodiesel by enzymatic hydrolysis followed by enzymatic esterification of the acid oil from macauba pulp.

- **Acid oil from macauba** → **Hydrolysis** → **Esterification** → **Biodiesel**

- **Vegetable oil obtained from** dormant castor seeds

- Lipase from *Rhizomucor miehei* in the form of dry fermented solid

- Babassu cake + *R. miehei*

- Use of solid wastes that are agro-industrial residues (oil cakes) for microbial growth and production of the lipases.

- Reducing costs (extraction, purification and immobilization).

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✓ Acidity → 10.5%
✓ Composition in fatty acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capric (C10:0)</td>
<td>0.1</td>
</tr>
<tr>
<td>Lauric (C12:0)</td>
<td>1.2</td>
</tr>
<tr>
<td>Miristic (C14:0)</td>
<td>0.4</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>19.1</td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>4.1</td>
</tr>
<tr>
<td>Estearic (C18:0)</td>
<td>1,3</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>53.7</td>
</tr>
<tr>
<td>Linoleic (C18:2)</td>
<td>18.8</td>
</tr>
<tr>
<td>Linolenic (C18:3n3)</td>
<td>1.3</td>
</tr>
</tbody>
</table>
RESULTS

ENZYMATIC HYDROLYSIS OF ACID OIL

Percentage of 99.6% of FFAs was attained in 24 h.

Hydrolysis reaction was carried out with high oil concentration (50% v/v), low concentration of a non-commercial lipase and without organic solvent and emulsifier.

Kinetic of hydrolysis of the acid oil from macauba catalyzed using 50% (v/v) oil and 50% (v/v) buffer.

Conditions of the reactions: VE: 2.5% (w/v) lipase, 0.1M sodium acetate buffer pH 4.0 at 30°C.
Lipomod: 1% (w/v) lipase, 0.1M Tris-HCl buffer pH 8.0 at 30°C.

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RESULTS
ENZYMATIC ESTERIFICATION OF FREE FATTY ACIDS

✔ Comparison of ethanol addition in steps and continuously
✔ Solvent-free medium

Conversions were similar for all conditions studied in 6h.

The stepwise ethanol addition in 0, 1 and 2 h was chosen for later studies.

With a final molar ratio ethanol:acid = 2:1 (1/6 of the total ethanol volume added at 0, 1 and 2 h and ½ added at 4 h) the conversion of 91% was achieved after 8 h.

Kinetic of esterification reaction of FFAs from macauba oil hydrolyzate with stepwise or continuous ethanol addition. The reaction was conducted with molar ratio ethanol:acid = 1:1 and 15.1 U of dry fermented solid per g of FFAs at 40 °C.

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RESULTS
COMPARISON OF THE FERMENTED SOLID WITH COMMERCIAL LIPASES

The kinetic of the reaction catalyzed by the fermented solid was similar to those obtained with commercial lipases.

- **Novozym 435**
  2510 US$/Kg
- **Lipozyme RM IM**
  847 US$/Kg
- **Fermented solid produced by SSF**
  567.54 US$/ton

Kinetic of esterification reactions of FFAs from macauba oil hydrolyzate with ethanol catalyzed by lyophilized fermented solid, Novozym 435 or Lipozyme RM IM. Ethanol was added at 0, 1 and 2 h (R ethanol:acid = 1:1) using 14 U of esterification activity per g of FFAs at 40 °C.
The fermented solid remained active after ten cycles and the values of conversion and ester yield remained above 95% of the initial values during eight cycles.

The fermented solid is highly stable and acts as a support that keeps the lipase adsorbed on its structure.

Effects of the lyophilized fermented solid reuse on fatty acids conversion and ester yield. Each reaction was conducted for 6 h, using macauba oil hydrolyzate and ethanol as substrates (ethanol added at 0, 1 and 2 h (R ethanol:acid = 1:1)) and 15.5 U of dry fermented solid per g of FFAs at 40 °C.

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RESULTS

BIODIESEL PRODUCTION AND CHARACTERIZATION

✓ The FFAs produced by hydrolysis of macauba oil were used in two consecutive esterification reactions with ethanol.

✓ After the first esterification reaction, the product showed an ester yield of 89.7% and an acidity of 6.45%.

✓ This product was used in a second esterification reaction, in order to consume the residual FFAs and reduce its acidity. After 24 h the acidity was reduced to 1.52%.

✓ The biodiesel produced was analyzed according to the standard methodologies published by ASTM (American Society for Testing and Materials) and ABNT (Associação Brasileira de Normas Técnicas – Brazilian Technical Standards Association).
## RESULTS

### BIODIESEL PRODUCTION AND CHARACTERIZATION

Resolution ANP Nº 14 (11/05/2012)

<table>
<thead>
<tr>
<th>Properties</th>
<th>Unity</th>
<th>Result</th>
<th>Min.</th>
<th>Max.</th>
<th>Método</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density 20°C</td>
<td>kg.(m³)⁻¹</td>
<td>872.2</td>
<td>850</td>
<td>900</td>
<td>ASTM D4052</td>
</tr>
<tr>
<td>Water content, max.</td>
<td>mg.Kg⁻¹</td>
<td>219</td>
<td></td>
<td>200</td>
<td>ASTM D6304</td>
</tr>
<tr>
<td>Kinematic viscosity 40°C</td>
<td>mm².s⁻¹</td>
<td>5.01</td>
<td>3.0</td>
<td>6.0</td>
<td>ASTM D445</td>
</tr>
<tr>
<td>Flash point, min.</td>
<td>°C</td>
<td>151</td>
<td>100</td>
<td></td>
<td>ASTM D93</td>
</tr>
<tr>
<td>Carbon residue, max.</td>
<td>wt. %</td>
<td>0.039</td>
<td></td>
<td>0.05</td>
<td>ASTM D4530</td>
</tr>
<tr>
<td>Oxidation stability 110°C</td>
<td>h</td>
<td>0.95</td>
<td>6</td>
<td>-</td>
<td>EN 14112</td>
</tr>
<tr>
<td>Ester content</td>
<td>wt. %</td>
<td>95.9</td>
<td>96.5*</td>
<td>-</td>
<td>EN 14103</td>
</tr>
<tr>
<td>Methanol or ethanol, max.</td>
<td>wt. %</td>
<td>0.32</td>
<td></td>
<td>0.2</td>
<td>EN 14110</td>
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<tr>
<td>Free glycerol, max.</td>
<td>wt. %</td>
<td>0.0058</td>
<td></td>
<td>0.20</td>
<td>ASTM D6584</td>
</tr>
<tr>
<td>Total glycerol, max.</td>
<td>wt. %</td>
<td>0.1024</td>
<td></td>
<td>0.25</td>
<td>ASTM D6584</td>
</tr>
<tr>
<td>Monoglycerides, max.</td>
<td>wt. %</td>
<td>0.1516</td>
<td></td>
<td>0.80</td>
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<tr>
<td>Diglycerides, max.</td>
<td>wt. %</td>
<td>0.3841</td>
<td></td>
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<tr>
<td>Triglycerides, max.</td>
<td>wt. %</td>
<td>0.0022</td>
<td></td>
<td>0.20</td>
<td>ASTM D6584</td>
</tr>
</tbody>
</table>
The production of cheap biocatalysts, the development of low cost and environmentally friendly process, and the exploration of alternative potential feedstocks are the gains of this study;

The enzyme/enzyme hydroesterification process using low-cost biocatalysts in both reactions and macauba acid oil as raw material is described for the first time in this work;
The use of this acid oil to biodiesel production does not compete with the food industry, allows a diversification of the oil crops used for biodiesel production in Brazil and provides the Social Seal to biodiesel producers that buy this oil from family farmers;

The resulting fuel properties met important Brazilian standards and the product can be employed as a blend. Considering a large-scale process, it could represent an important contribution for regional use of resources and alternative vegetable oil crops such as macauba;

The enzyme/enzyme hydroesterification process described in this study appears to be a promising alternative to the traditional process of biodiesel production and can contribute to make the enzymatic biodiesel economically feasible.
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THANK YOU!

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