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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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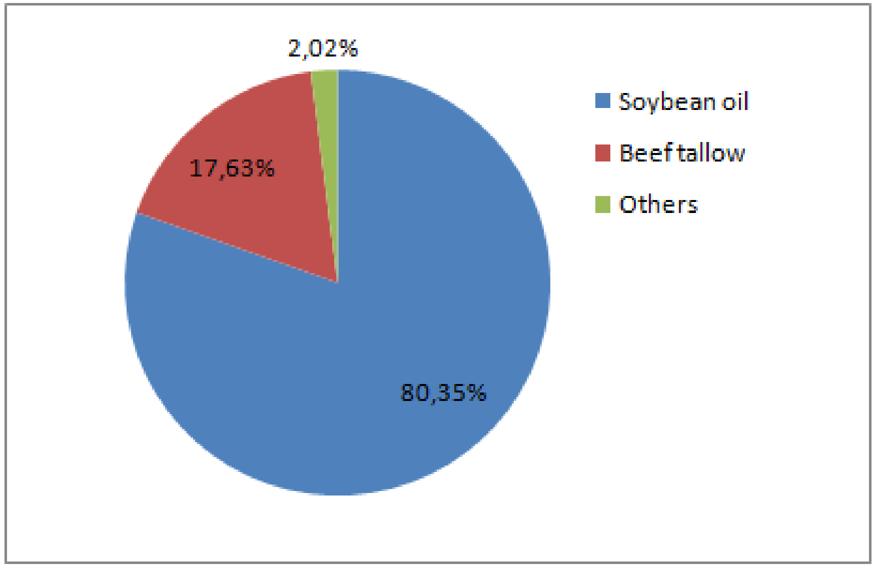
INTRODUCTION

BIODIESEL

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 m^3 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

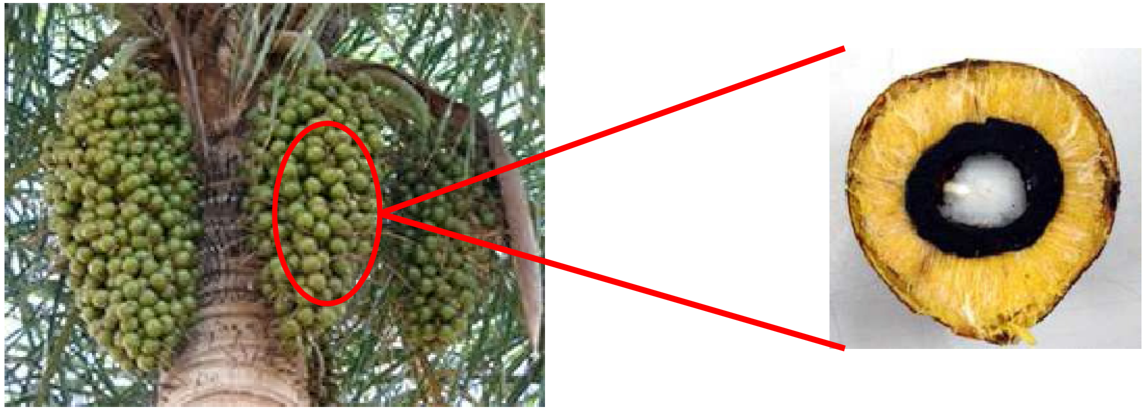
INTRODUCTION

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.

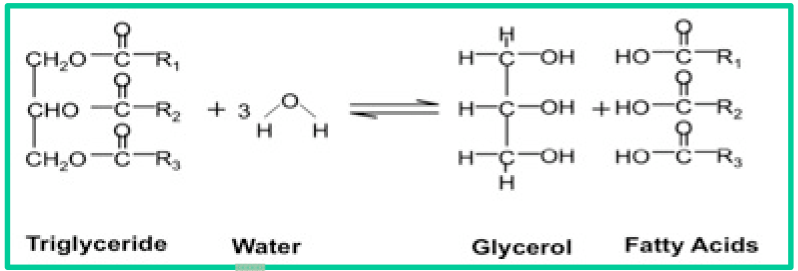


INTRODUCTION

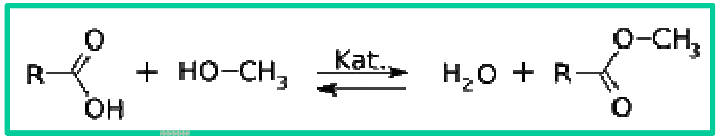
ENZYME/ENZYME HYDROESTERIFICATION ROUTE

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)



$$R{-}COOH + HO{-}CH_3 \xrightleftharpoons{Kat.} H_2O + R{-}COO{-}CH_3$$

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!

AIM

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**

VE obtained from dormant castor seeds ↑ Hydrolysis

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

Babassu cake + *R. miehei* ↑ Lipase from *Rhizomucor miehei* in the form of dry fermented solid

✓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).

RESULTS

CHARACTERIZATION OF THE ACID OIL

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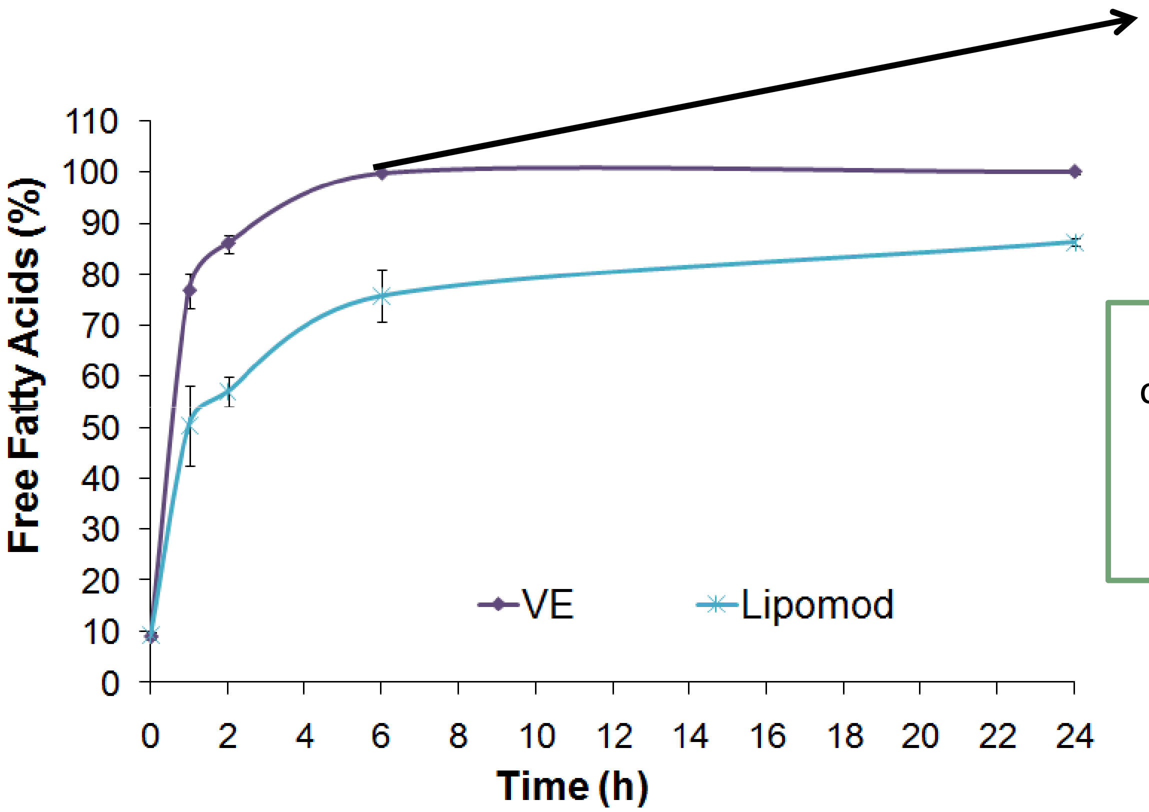
- ✓Acidity → 10.5%
- ✓Composition in fatty acids

Fatty acid	Percentage
Capric (C10:0)	0.1
Lauric (C12:0)	1.2
Miristic (C14:0)	0.4
Palmitic (C16:0)	19.1
Palmitoleic (C16:1)	4.1
Estearic (C18:0)	1,3
Oleic (C18:1)	53.7
Linoleic (C18:2)	18.8
Linolenic (C18:3n3)	1.3

RESULTS

ENZYMATIC HYDROLYSIS OF ACID OIL



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

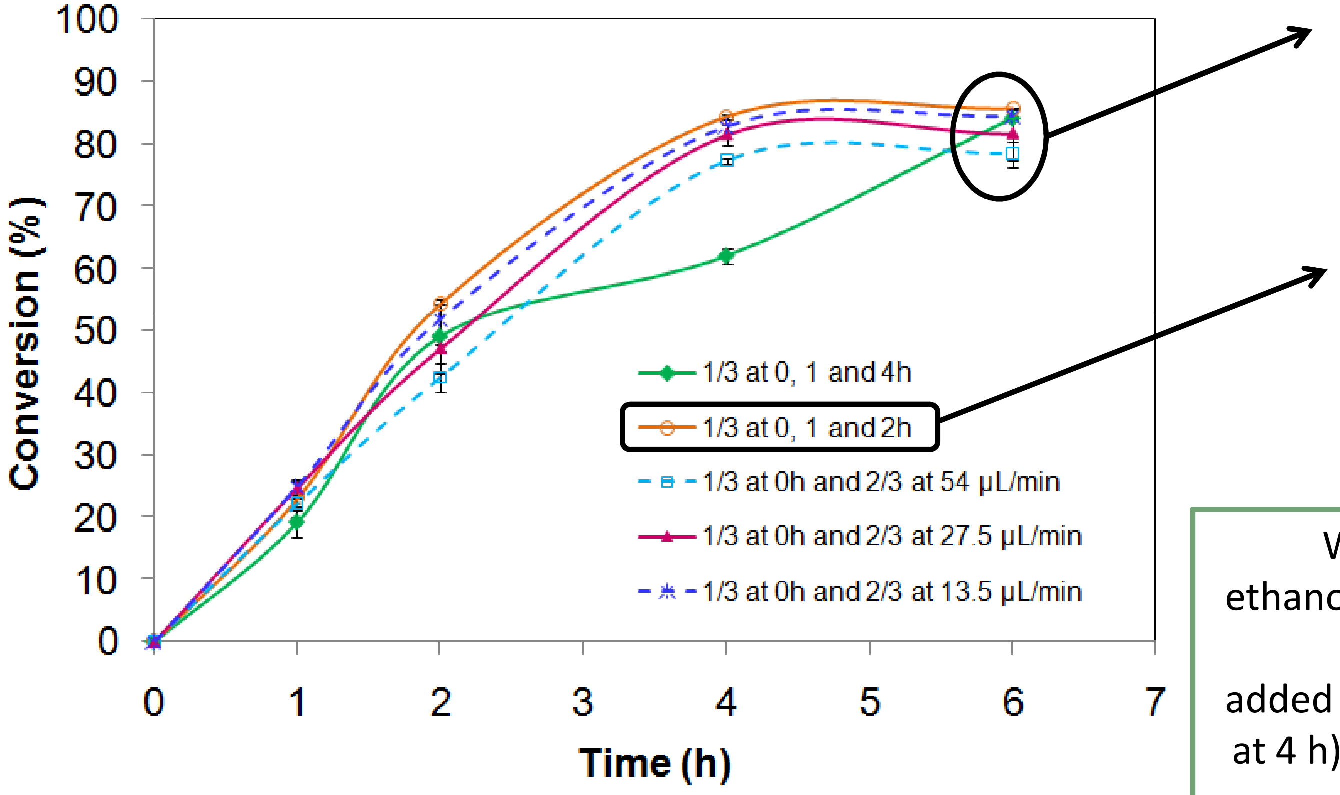
Hydrolysis reaction was carried out with high oil concentration (50% v/v), low concentration of a non-commercial lipase and without organic solvent and emulsifier.

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.

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.

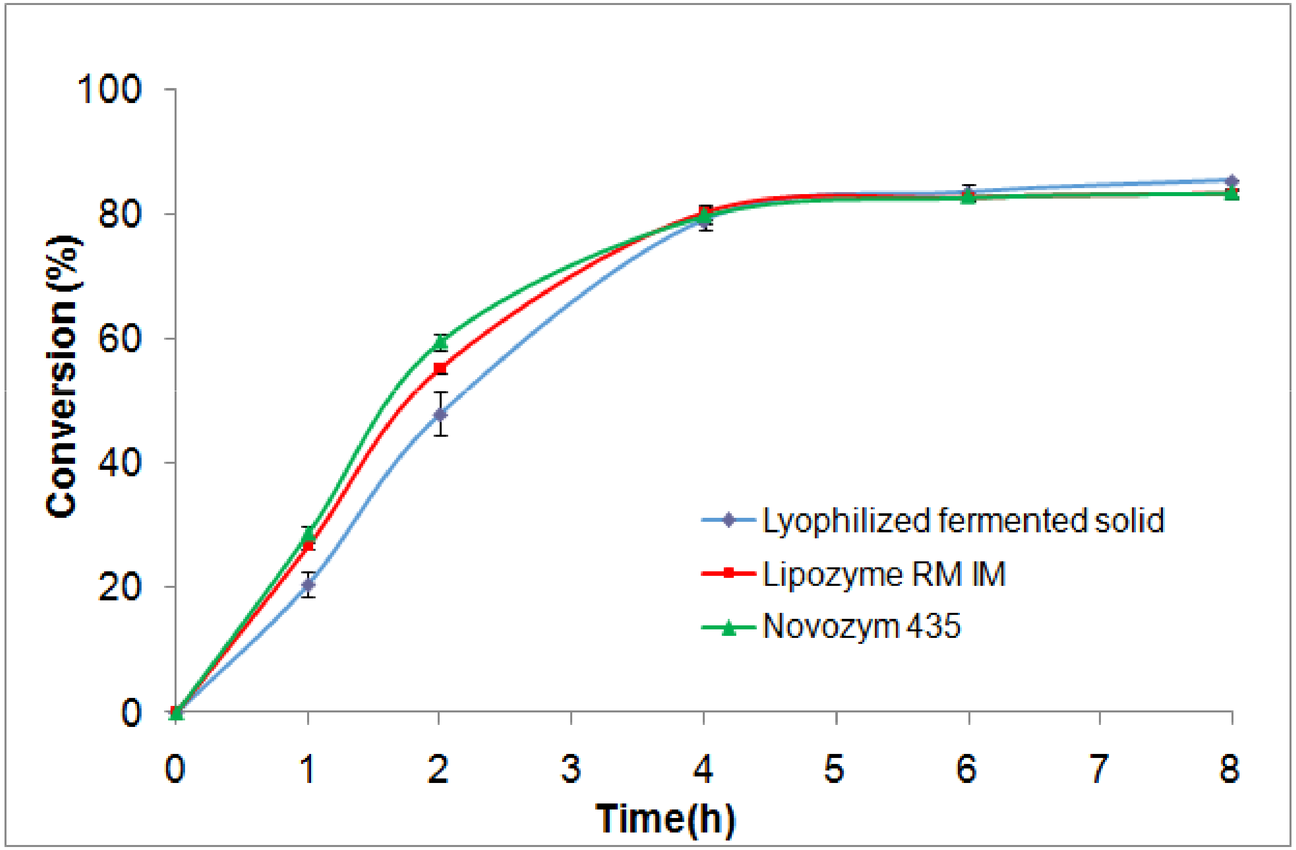
The stepwise ethanol addition in 0, 1 and 2 h was chosen for later studies.

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.

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.

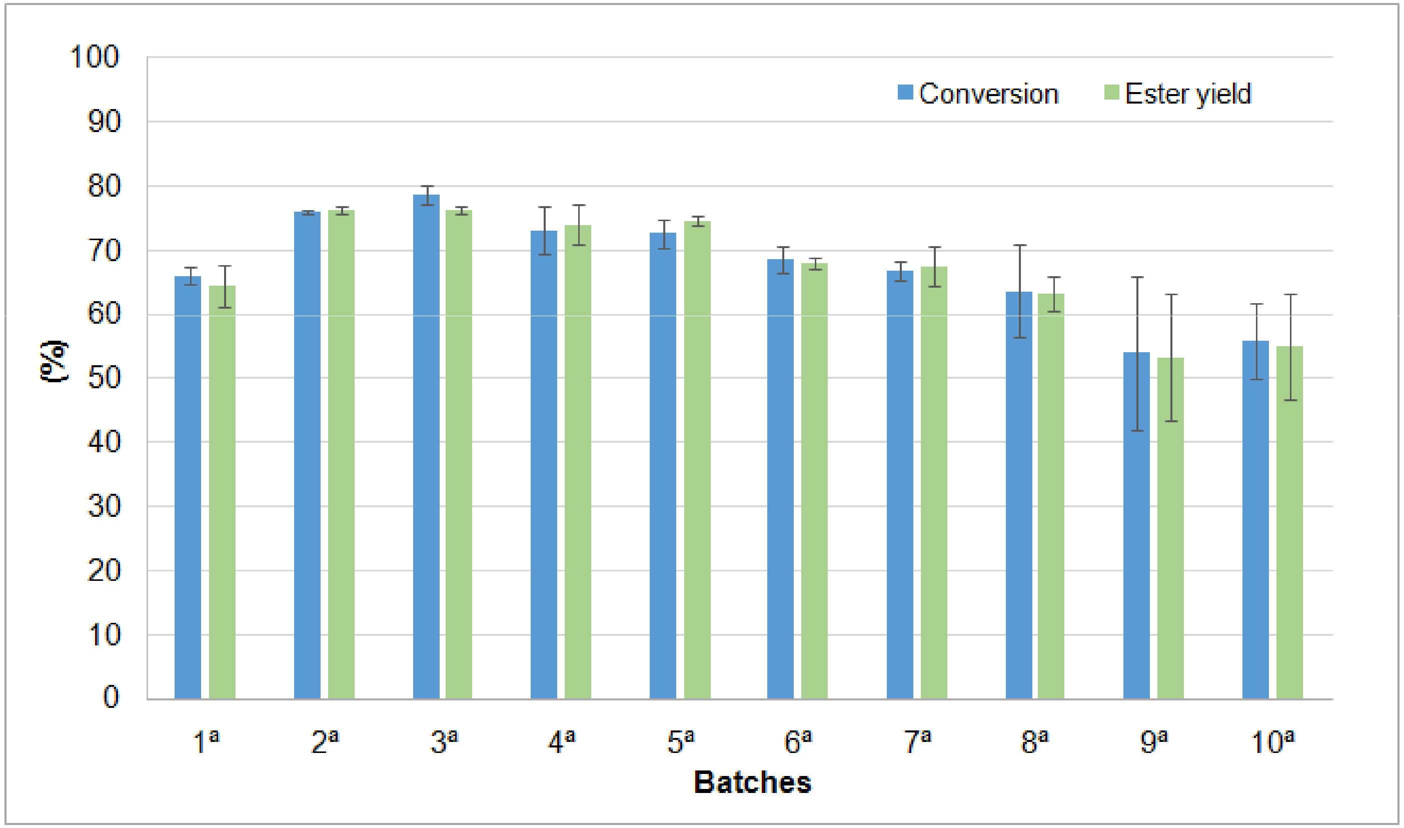
- **Novozym 435**
  2510 US$/Kg
- **Lipozyme RM IM**
  847 US$/Kg
- **Fermented solid produced by SSF**
  567.54 US$/ton

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.

RESULTS

OPERATIONAL STABILITY OF THE FERMENTED SOLID IN BATCH REACTOR

✓The reuse of fermented solids was investigated in successive esterification batch reactions of 6 h.



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

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✓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

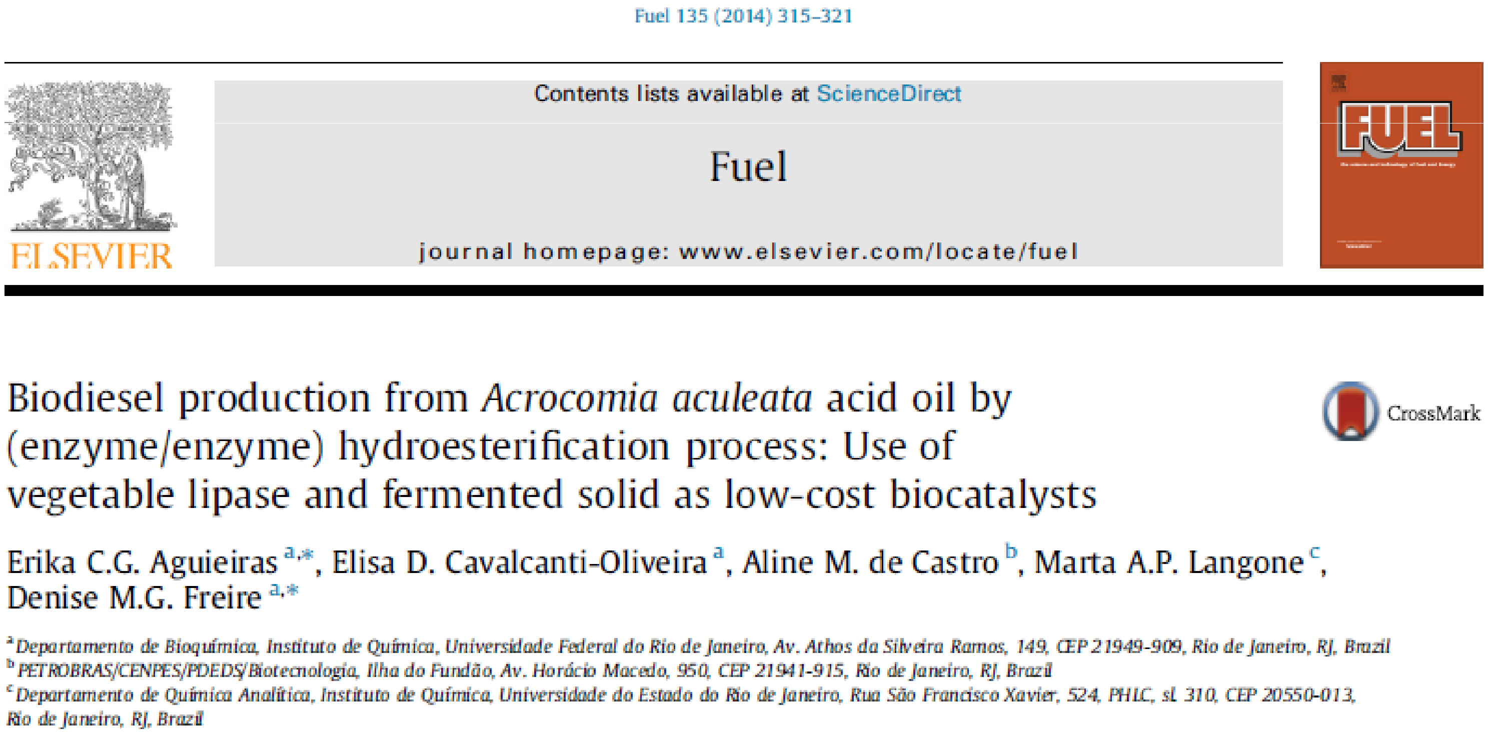
BIODIESEL PRODUCTION AND CHARACTERIZATION

			Resolution ANP Nº 14 (11/05/2012)		
Properties	Unity	Result	Min.	Max.	Método
Density 20°C	kg.$(m^3)^{-1}$	872.2	850	900	ASTM D4052
Water content, max.	mg.Kg^{-1}	219		200	ASTM D6304
Kinematic viscosity 40°C	$mm^2.s^{-1}$	5.01	3.0	6.0	ASTM D445
Flash point, min.	ºC	151	100		ASTM D93
Carbon residue, max.	wt. %	0.039		0.05	ASTM D4530
Oxidation stability 110°C	h	0.95	6	-	EN 14112
Ester content	wt. %	95.9	96.5*	-	EN 14103
Methanol or ethanol, max.	wt. %	0.32	-	0.2	EN 14110
Free glycerol, max.	wt. %	0.0058	-	0.20	ASTM D6584
Total glycerol, max.	wt. %	0.1024	-	0.25	ASTM D6584
Monoglycerides, max.	wt. %	0.1516	-	0.80	ASTM D6584
Diglycerides, max.	wt. %	0.3841	-	0.20	ASTM D6584
Triglycerides, max.	wt. %	0.0022	-	0.20	ASTM D6584

CONCLUSIONS

✓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;



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CONCLUSIONS

✓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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