Biofuel similar to biodiesel obtained by using a lipase from *Rhizopus Oryzae*, optimized by response surface methodology

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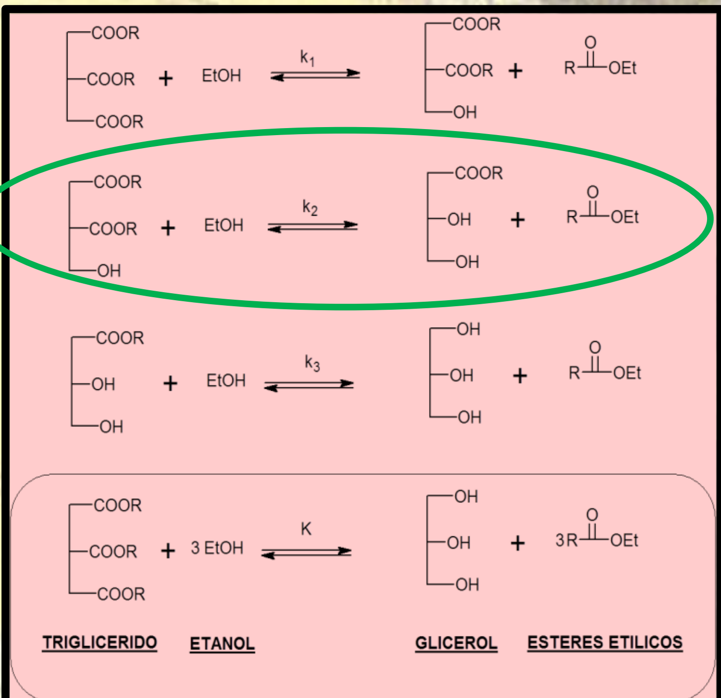
1. INTRODUCTION:

1.1. BIODIESEL (FAME/FAEE) vs. BIOFUELS (Bioesthers: FAEE+MG)

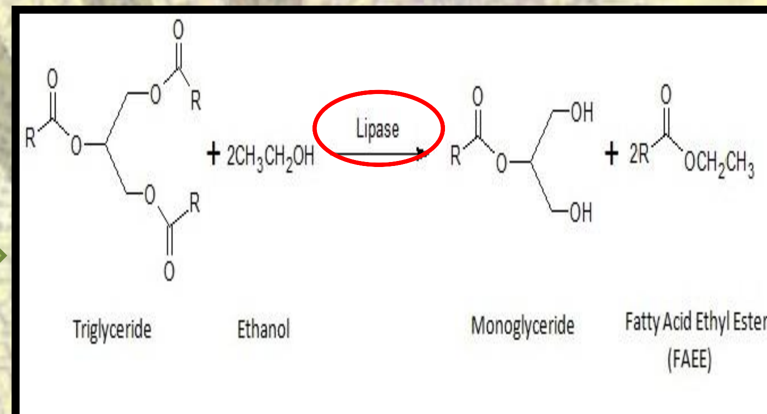
1.2. Alcoholysis reaction of TG:



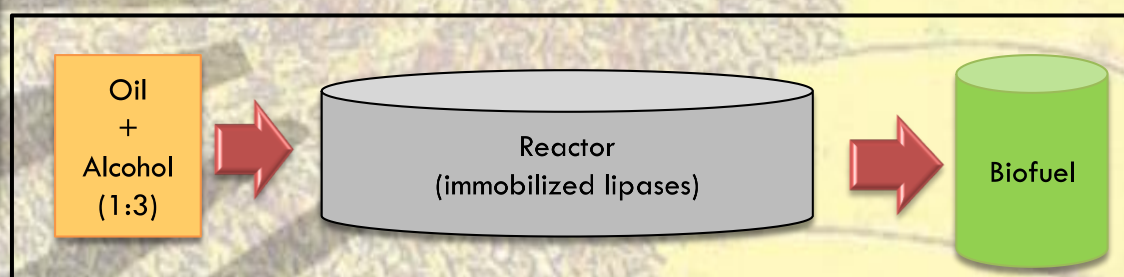
Biodiesel: FAME (EN14214)

1,3-Selective…

1.3. Enzymatic alcoholysis : "ECODIESEL®"



PPL/ WILD & COMMERCIAL MICROBIAL LIPASES



2. OBJECTIVES:

- ✓ Evaluation of **BIOLIPASE-R®**, which is a multipurpose alimentary additive from Biocon®-Spain that consist in a low cost powdered enzyme preparation containing obtained lipases from a strain of ***Rhizopus oryzae (ROL***) capable of performing useful **transesterification reactions** in the synthesis of an alternative second generation biodiesel.

- ✓ Obtaining a **free glycerol biodiesel** with similar properties to conventional biodiesel and with **a suitable viscosity** for its use in diesel motors, pure or mixed with fossil diesel.

- ✓ Optimize several crucial ethanolysis reaction parameters, firstly **water content** and **amount of lipase**, and then it is applied with these optimum values a multi-factorial design of experiments and **response surface methodology for temperature, oil/ethanol volumetric ratio and pH.**

- ✓ The Applying of a fast and reliable analytical method, based on **gas chromatography** , to characterize products obtained in the partial alcoholysis reaction of triglycerides (mixtures **of FAEEs, MGs and DGs).**

3.EXPERIMENTAL:

3.1. Ethanolysis Reaction Device:

Figure 3.1.Reaction conditions: the temperature range between 20-40 °C and for reaction times of 60 minutes using 12 mL of sunflower oil with variable EtOH amounts (1.75-3.5 ml) in a 25 mL flask with magnetic stirring higher than 300 rpm.

3.2. Statisical experimental design to evaluate reaction parameters: ANOVA (Tª, R., pH) vs. OVAT (a_w, Lipase amount)

Parameters	Unit	Levels		
		-1	0	1
Temperature	°C	20	30	40
Oil/Ethanol ratio (v/v)	mL/mL	12/1,75	-	12/3.5
pH	µL (NaOH 10N)	8 /12.5	10/5	12/50

Table 3.1. Process parameters in factorial design: coded and actual values.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_0 x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} x_i x_j$$

Stat Graphics version XV.I

3.EXPERIMENTAL:

3.3. Biofuel composition determination by GC & Viscosity measurements...

Fig. 3.2. **Varian 430-GC**, connected to a HT5 capillary column with a flame ionization detector (**FID**) at 450 °C and **splitless injection** at 350 °C. **Helium** is used as carrier gas, with a flow of 1.5 ml/min. It has been applied a heating ramp from 90 °C to 200 °C at a rate of 7 °C/min, followed by another ramp from 200 °C to 360 °C at a rate of 15 ºC/min, maintaining the temperature of the oven at 360 °C for 10 minutes.

3.4. Comparative chromatograms of standardized reaction products :

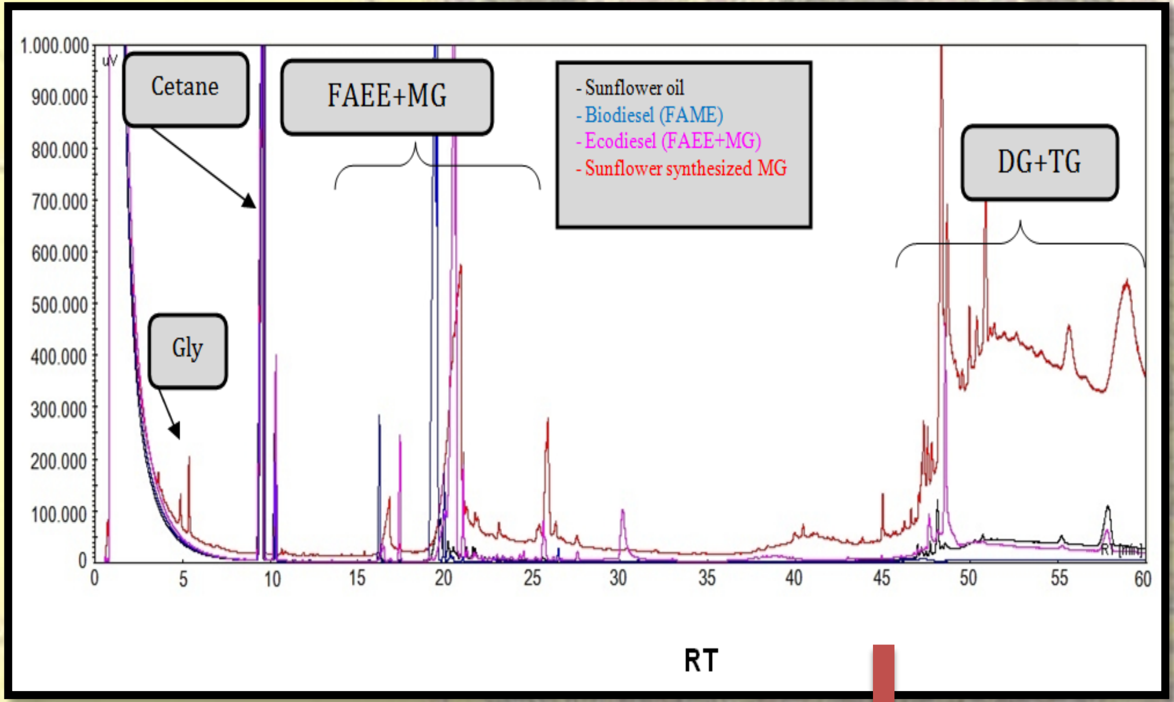


Fig. 3.3. Superimposed chromatograms of sunflower oil (black), as well as obtained chromatograms in the alcoholysis of sunflower oil with methanol (**FAME**), ethanol (**FAEE**) and glycerol (MG) corresponding to blue, pink and red respectively.

Internal Standard: CETANE (n-hexadecane)

- ✓ To cuantify DG and TG that are not determinate by GC.
- ✓ Reference Compound: Cetane number for diesel.

4 . RESULTS & DISCUSSION:

4.1. Effect of water content

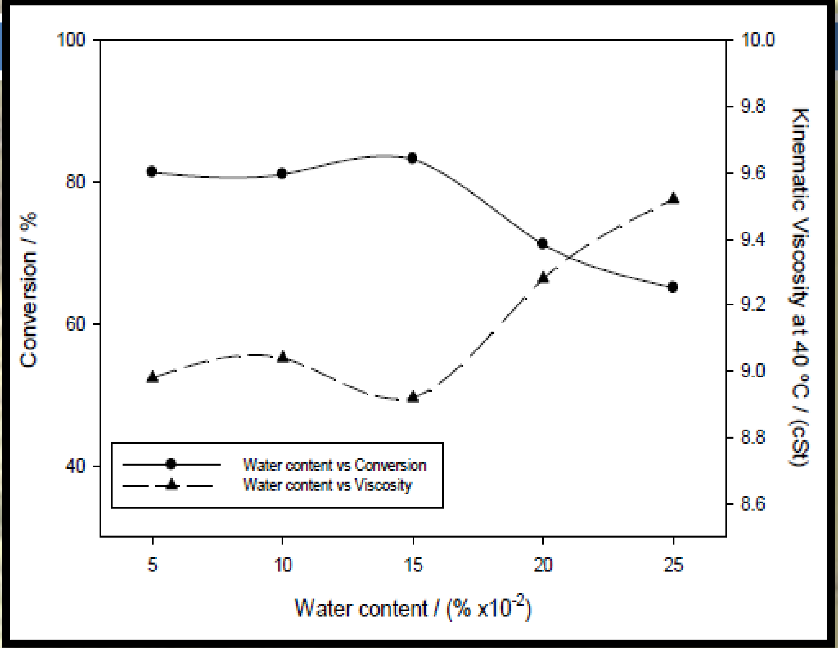


Fig. 4.1. Influence of water content on ethanolysis reaction yield (conversion and kinematic viscosities) .

4.2. Effect of the lipase quantity

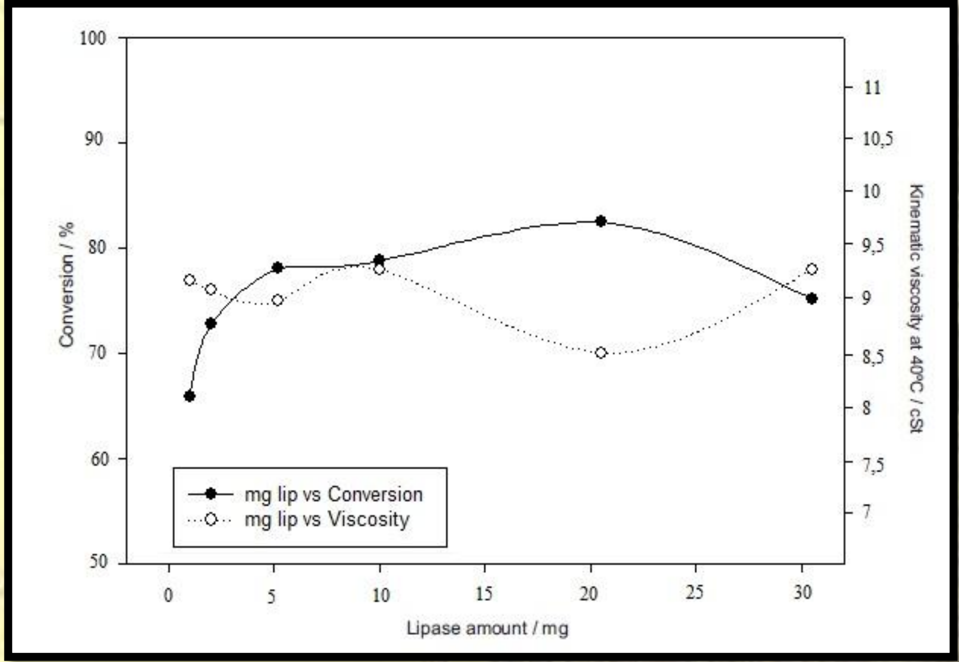


Fig. 4.2. Influence of the quantity of lipase on ethanolysis reaction yield (conversion and kinematic viscosities)

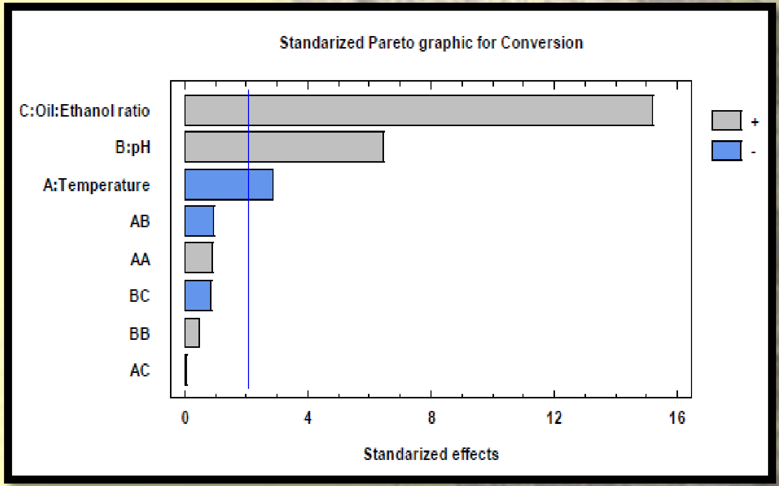
4 . RESULTS & DISCUSSION:

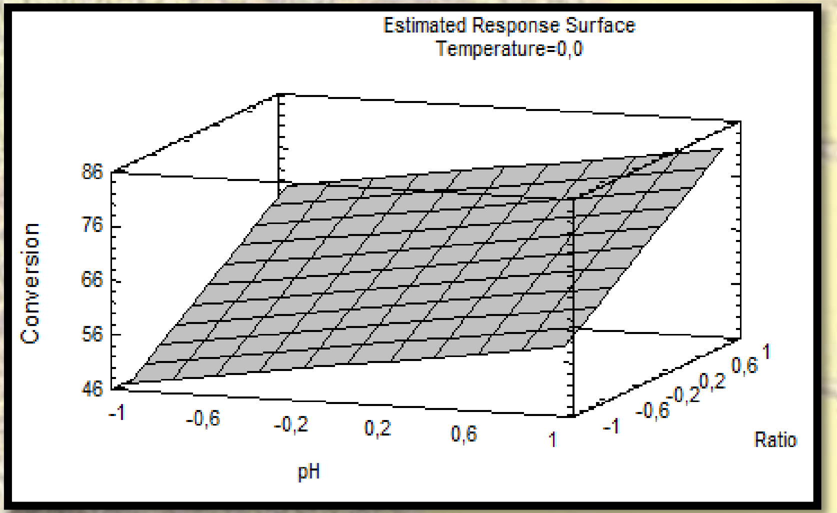
4.3. Analysis of variance (ANOVA) and optimization of the reaction parameters by RSM

4.3.1. Analysis of variance (ANOVA) for Conversion

R^2 = 91,5878 %; R^2 (adj.) = 89,0953 %

$$[Conversion\ (\%) = 64{,}0306 - 2{,}60 \times T + 5{,}90 \times pH + 11{,}36 \times R]$$





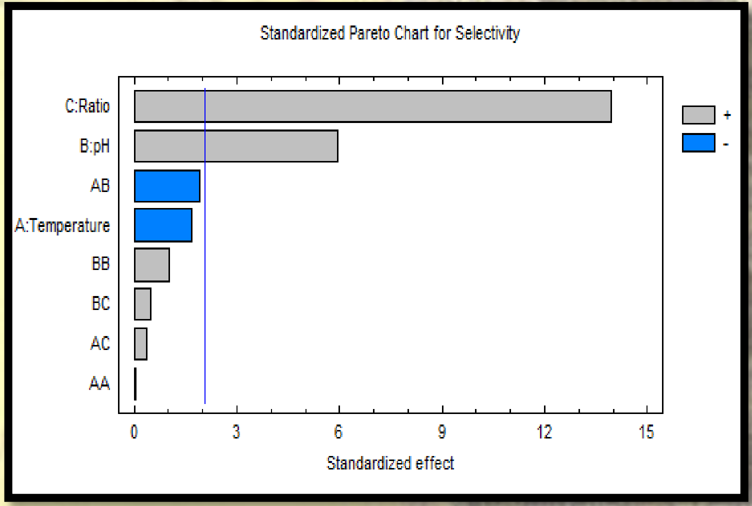
4 . RESULTS & DISCUSSION:

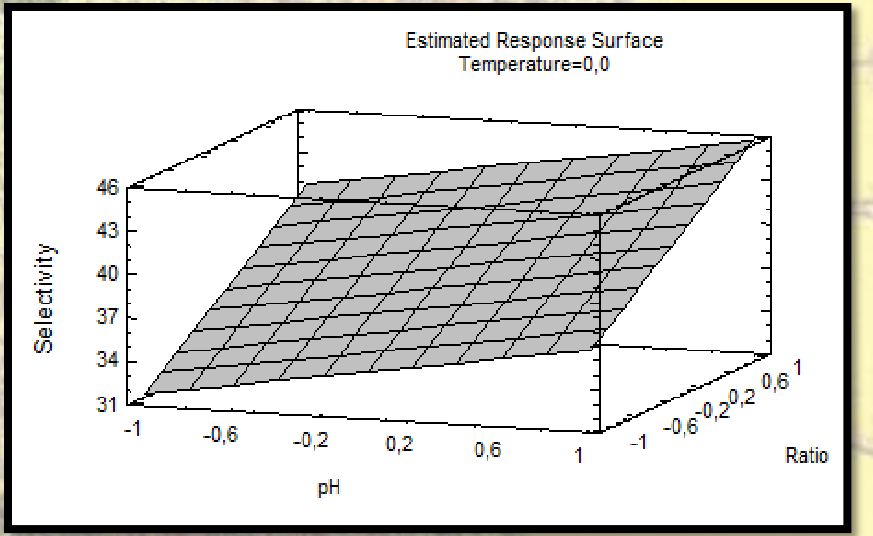
4.3. Analysis of variance (ANOVA) and optimization of the reaction parameters by RSM

4.3.2. Analysis of variance (ANOVA) for Selectivity

$R^2 = 90,1257$ %; R^2 (adj.) = 87,2 %

$$[Selectivity(\%) = 38,76 + 2,43 \times pH + 4,66 \times R]$$





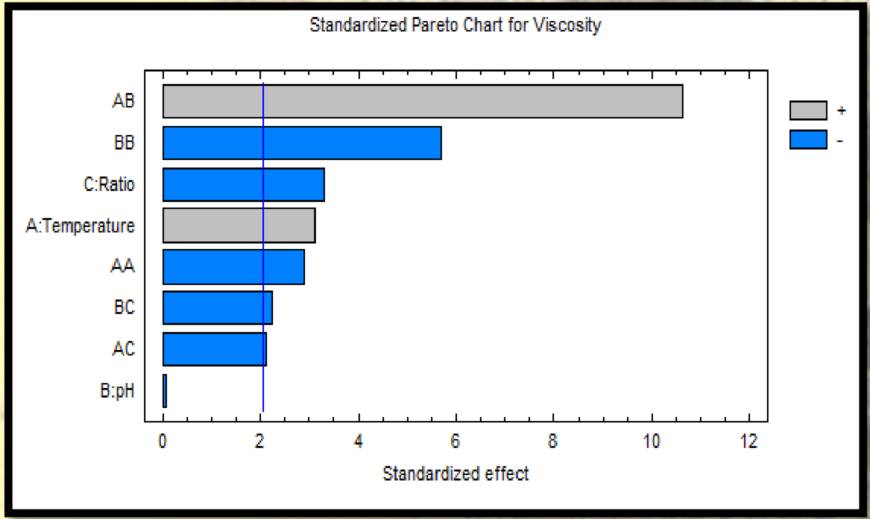
4 . RESULTS & DISCUSSION:

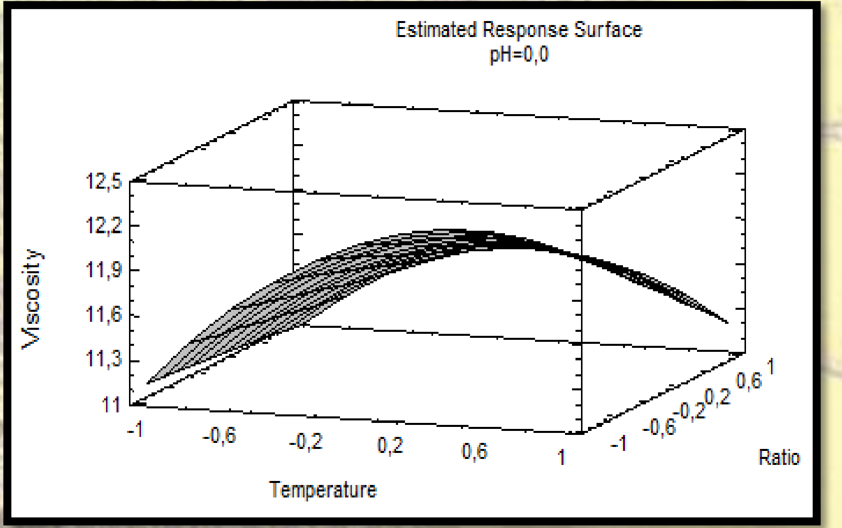
4.3. Analysis of variance (ANOVA) and optimization of the reaction parameters by RSM

4.3.3. Analysis of variance (ANOVA) for Viscosity

R^2 = 87,6582 %; R^2 (adj.) = 84,0013 %

$$Viscosity\ (cSt) = 11{,}8944 + 0{,}3125 * T - 0{,}2694 \times R + 0{,}5042 \times T^2 + 1{,}306 \times T \times pH - 0{,}2125 \times T \times R - 0{,}9917 \times pH^2 - 0{,}225 \times pH \times R$$





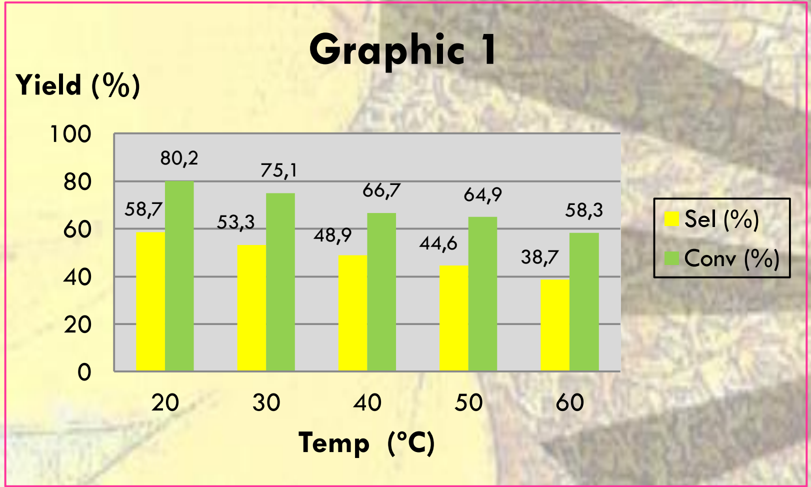
4 . RESULTS & DISCUSSION:

4.4. Experimental validation of proposed model

Temp °C	pH	Oil/ethanol ratio	Conversion (%)		Selectivity (%)	
			Exp.	Adj.	Exp.	Adj.
25	12	12/2.9	68.8	72.7	39.5	41.5
30	10	12/2.75	63.7	66.8	42.3	39.4
50	8	12/3.3	70.9	68.1	44.7	40.9

Table 4.1. Validation experiments of proposed models for the enzyme Biolipase-R.

4.5. Influence of temperature (OVAT)



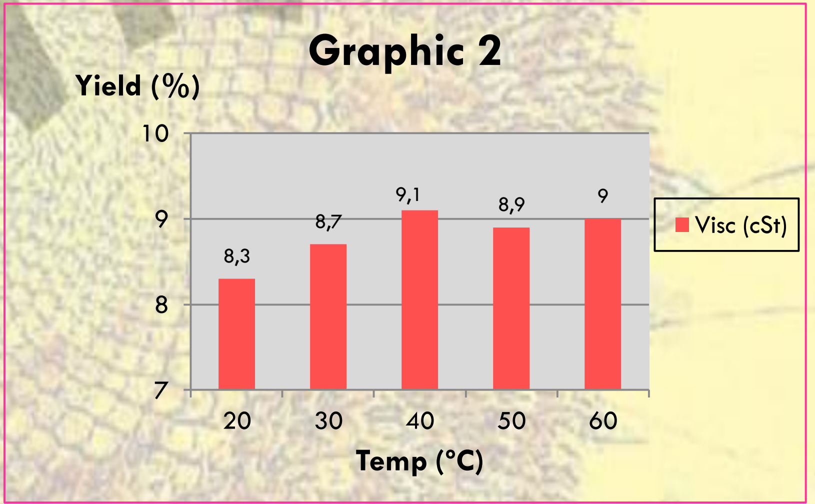


Fig. 4.3. Influence of the temperature on ethanolysis reaction yield (conversion, selectivity in **Graphic 1** and kinematic viscosities in **Graphic 2**).

4 . CONCLUSIONS :

✓Results show that **substrate molar ratio of ethanol to sunflower oil, pH and reaction temperature, as well as the water content and biocatalyst amount**, have a significant effect on the percentage of **ethanolysis reaction yield** (conversion, selectivity and cinematic viscosity).

✓On the basis of **RSM analysis**, we found that operates optimally with a **water conte**nt of the reaction medium of **0.15 %, 0.05 - 0.1 % lipase by weight relative to the weight of oil used, 20 °C, volume ratio (ml/ml) oil/ethanol 12/3.5 and pH 12** (by addition of **50 µl** of **10 N NaOH dissolution).**

✓It is shown the demonstration of the **viability of BIOLIPASE-R® (R.O.L)** to obtain an alternative biodiesel-like biofuel that integrates glycerine (**"ECODIESEL"**) that can be used in different blends with diesel fuel, without anymore separation or purification process.

✓This biofuel can be **economically viable and environmentally sustainable** since by using a low cost and industrially available lipase, with high yield at very short reaction times (less than 1h) and under soft reaction conditions.

✓The Tuning and application of an analytical method for the composition determination of biofuel that integrates MG, on **gas chromatography** based.

✓These results will be also used for **further immobilization studies**.

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Thanks for your interest and to the conference organizers, to the scientific Committee for inviting me to make this on line communication in the first International e-conference on Energies...

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