CYNARA CARDUNCULUS AS AN ALTERNATIVE CROP FOR BIODIESEL PRODUCTION

DISSERTATION PRESENTED BY

JORGELINA CECILIA PASQUALINO

TO OBTAIN THE DEGREE:
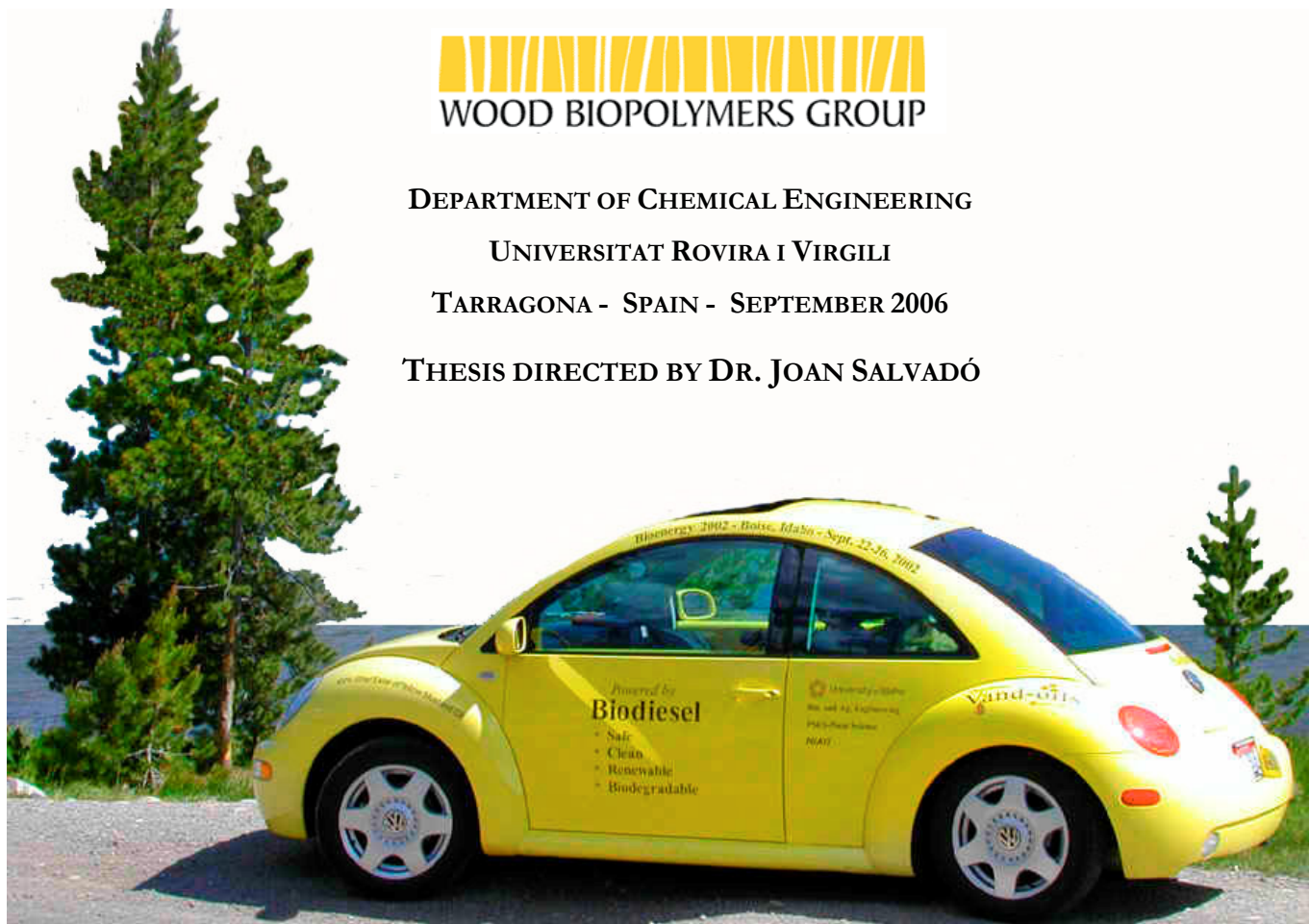
DOCTOR IN CHEMICAL ENGINEERING

WOOD BIOPOLYMERS GROUP

DEPARTMENT OF CHEMICAL ENGINEERING

UNIVERSITAT ROVIRA I VIRGILI

TARRAGONA - SPAIN - SEPTEMBER 2006

THESIS DIRECTED BY DR. JOAN SALVADÓ

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CYNARA CARDUNCULUS AS AN ALTERNATIVE CROP FOR BIODIESEL PRODUCTION

Para optar al grado de Doctor en Ingeniería Química por la Universitat Rovira i Virgili

HACE CONSTAR:

Que el citado trabajo es original y que todos los resultados presentados y los análisis realizados son fruto de su investigación.

Y para vuestro conocimiento y los efectos que correspondan, firma este documento

Tarragona, 4 de septiembre de 2006

Joan Salvadó i Rovira

Yo, vine creciendo y me forjé

cual mi generación distinta a la de ayer.

(...)

Soy como quisieron ser pero tratando de ser yo,

ni menos mal, pero en verdad ni menos bien.

No ha sido fácil tener

una opinión que haga valer

mi vocación, mi libertad para escoger.

(...)

Yo, yo sólo tengo la razón

de quien quisiera ser mejor de lo que ayer.

(...)

Sufre conmigo el error que cometeré

goza también lo que de bien

se ha de lograr sin pretender.

Sube conmigo a encontrar el escalón

que evocaré para llegar

a ese lugar que un día soñé.

PABLO MILANÉS, NO HA SIDO FÁCIL, 1984

AGRADECIMIENTOS

Me gustaría empezar este trabajo con unas palabras de agradecimiento a todas aquellas personas que de alguna manera me ayudaron a alcanzar este momento.

Sé que esta es la parte de la tesis que más gente leerá, y probablemente la única que lean algunos de ustedes, y por tanto espero no olvidarme de nadie.

En primer lugar quiero agradecer al Doctor Joan Salvadó, porque no solo fue el director este trabajo sino que además me brindó su apoyo durante estos 4 años, dedicando ideas, tiempo y paciencia, que no es poco. Porque no solo me enseñó el camino a seguir sino que también me dejó experimentar por mi cuenta, aunque me equivocara. Porque no solo ha estado pendiente de mi trabajo sino también de mi vida, mi futuro, mi familia, y hacer de ese modo que mi estadía acá sea mucho más fácil. Por confiar en mi. Gracias Joan.

También quisiera agradecer a los miembros del tribunal de tesis, por evaluar este trabajo. Gracias Dr. Manuel Bao Iglesias, Dr. Domingo Cantero Moreno, Dr. José Antonio Reina Lozano, Dra. Paloma Manzanares Secades, Dr. Daniel Montané i Calaf, Dra. Mercedes Ballesteros Perdices y Dra. Marta Giamberini.

El grupo de investigación Biopolímeros Vegetales, merece un doble agradecimiento, como grupo de trabajo y como grupo de aventuras. En cuanto al trabajo, quiero agradecerles por el apoyo en el laboratorio, por compartir el material y el tiempo que pasamos en él, por los comentarios aportados en los ensayos de las presentaciones, las críticas, las ideas, las sugerencias, y en definitiva por hacer el trabajo más ameno. En cuanto a las aventuras, quiero agradecerles por las reuniones gastronómicas que hemos compartido en estos años, los pica-picas, paellas, cabritos, calçotadas y demás experimentos culinarios, a veces también acompañados de un paseo. Gracias a los profesores del grupo, Joan Salvadó, Daniel Montané, Ricard García, Xavier Farriol, Francesc Ferrando y Marta Giamberini, y a los becarios, Nour Edine, Freddy, Camilo, Debora, Iü, José Antonio, Vanessa, Luizildo, Carles, Dawit, Baltazar y Pepa. Y también sumo a este agradecimiento a quienes han dejado el grupo en este tiempo, Vanessa, Guillermo, Lourdes, Xiao, Daiyong, Cati, Modest, Anna, con quienes he compartido parte de este tiempo y de quienes espero no olvidar a nadie.

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lejos de casa ni me sienta sola nunca. Gracias a esos amigos que han compartido esos momentos en los que uno necesita a alguien a su lado. Gracias a esos amigos que han compartido mis cumpleaños y con quienes he brindado en Navidad y en Año Nuevo. Gracias a los amigos de la hora del almuerzo y del café, y a los del cine en casa, las reuniones, las noches de Trivial, el cine, los viajes, y una infinidad de momentos compartidos. Gracias a los amigos con los que además de los años pasados espero compartir más momentos en el futuro. No puedo nombrarlos a todos porque seguramente me olvidaría de muchos, pero hay algunos a quienes quisiera nombrar. Gracias Deby por los ya casi 10 años de amistad, por el pasado, el presente y el futuro que espero sigamos compartiendo, por estar siempre ahí, por los viajes, congresos, el proyecto final, los años de convivencia, gracias por tu amistad. Gracias Luizildo, compañero de viajes, conciertos, navidades, nevadas, hospitales, por ser el vecino, el amigo y el hijo mimado. Gracias Carmelo por ser un gran amigo, compañero de aventuras, y también hijo consentido. Gracias María Eugenia y Pedro, Alicia, José Antonio, Tere y Dieguito, Marelys, Diego y Ana Mercedes, Carles, Pepa, Freddy, Alexandre, Isabela, Alfredo, Camilo, Irama, Maretva, Laura, Vivi, Saravana, Carlos, Guillermo, Lulú, Yuvisa, Paola, Alejandra, Iosvani, Anna, Flor y todos los que forman parte de la gran familia becaria. y que me han hecho más felices estos años.

Porque para llegar hasta acá han pasado muchas cosas, quisiera agradecer a mis compañeros de estudios previos al doctorado, y actuales amigos. Gracias a Vale por ser mi primer compañera de banco en el colegio, porque nos conocemos desde los 6 años y mantenemos el contacto, porque tu familia es como si fuera la mía, por los mails, los encuentros en Buenos Aires, la ayuda en el trámite de la VISA, gracias amiguita. Gracias a las "tochas", Vero, Lule, Vale, Ailin y Vane, por animarse a ser mis primeras alumnas de química, física y matemática, porque crecimos juntas, por todos los secretos compartidos, los momentos vividos y los reencuentros. Gracias a Deby, Lucre y Mariana, compañeras de aventuras, tantos años en la universidad, tantas horas estudiando juntas, compañeras de proyecto final, pero sobretodo grandes amigas. Gracias a los compañeros de estudios, de congresos, de recibódromo, de años de amistad y momentos compartidos, Laura, Sol, Silvana, Gustavo, Hernán, Ezequiel, Agus, Natalia, Adrián, Lili, y María. Gracias Laura, Silvana y Gustavo por los reencuentros a este lado del charco.

Quiero recordar también a mis profesores de la Universidad Nacional de Mar del Plata, en particular a Patricia Haure, gracias a quien establecí el contacto con esta universidad.

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SUMMARY

Biodiesel is a renewable fuel obtained from vegetable oils or animal fats. Its properties are similar to that of fossil diesel fuel, allowing its use for diesel engines, both pure and in mixtures with fossil diesel fuel. Biodiesel is obtained from the transesterification reaction, where the triglycerides contained in the oil or fat react with a short chain alcohol in presence of a catalyst. The products of the reaction are biodiesel and glycerol, obtained in two separated phases. The alcohols used in the reaction are usually methanol and ethanol. Alkaline catalysts, usually NaOH and KOH are the most commonly used, though alcoxides, inorganic acids and lipases. Traditional raw materials for biodiesel production are the oils of rapeseed, sunflower, soybean and palm. However, some alternative raw materials such as animal fats and recycled oils, are also used.

This thesis is focused on the use of *Cynara cardunculus* oil. *Cynara cardunculus*, also known as wild cardoon or Castilian cardoon, is a non-domesticated robust perennial plant, from the family of artichoke. It is well adapted to the Mediterranean weather. The maximum production reaches 30-35 tons/ha per year, with about 2 tons of seeds. The seeds contain up to 25 % oil, with a similar composition to sunflower oil. Thus, *Cynara cardunculus* cultivation may represent an alternative for abandoned cropland and a good candidate as renewable energy source and biodiesel production.

The present work is divided into three main parts:

➢ A general introduction

➢ The production of biodiesel from unrefined Cynara cardunculus oil

➢ The characterisation of the mixtures of biodiesel with fossil diesel fuel

➢ The biodegradability behaviour of biodiesel and its mixtures with fossil derived fuels

In the first part, the need of renewable energies, biofuels and specially biodiesel's use, was explained. The history of diesel motors and biodiesel was detailed, together with the actual situation, a comparison to conventional fossil diesel fuel and the main production characteristics.

In the second part, the reaction conditions where optimised for the transesterification of unrefined Cynara cardunculus oil. The best operation conditions where obtained for the reaction at 60ºC using methanol in a molar ratio of 6:1 with respect to oil, and 1% NaOH as catalyst.

The product obtained was characterised, determining the variations in viscosity and the content in methyl esters, glycerol, mono glycerides, diglycerides and triglycerides. The acid value of the original oil (11.8 mgKOH/g) was higher than the values recommended for alkaline transesterification (1-2 mgKOH/g), though for some reaction conditions the results were acceptable. However, most of the conditions experimented resulted in the formation of soaps and gels, and in some cases the ester and glycerol phases were not clearly separated. This results reflect the need of a preesterification step in order to reduce the acid value of the oil.

The preesterification step was optimised using different reaction temperatures, catalyst and methanol concentrations and reaction times. The best results were obtained for the reactions conducted at 60ºC, using a 6:1 methanol to oil molar ratio and 0.5% sulphuric acid as catalyst.

Finally, the oil was pre-treated using the best conditions for the preesterification, reducing the acid value to less than 1mgKOH/g. An additional degumming step was used in order to remove the phospholipids present in the oil.

The transesterification of the pre-treated oil was optimised and the results were compared to the previous ones without the use of pre-treatment. The ester yield increased with the pre-treatment and the ester and glycerol phases were clearly separated in most of the cases, showing the advantages of the degumming and preesterification steps.

The third part of the work was the characterisation of the mixtures of biodiesel and diesel fuel using standard specifications for diesel fuel. European specific normatives for both biodiesel (EN 14214) and fossil diesel fuel (EN 590) are detailed, together with their requirements and test methods. Mixtures of both fuel at different proportions, were analysed according to EN 590. All the analytical procedures were compared for diesel fuel and biodiesel. For some of the properties correlations for the mixtures were determined. In some cases experiments were needed to determine the behaviour of the mixtures. There are some properties of the mixtures than can limit the amount of biodiesel allowed in the

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mixture in order to satisfy the specifications for diesel fuel, such as density, viscosity, distillation, oxidative stability and cold weather properties. It was detected the need of specific analytical methods and requirements for the determination of the oxidative stability and the cetane index of the mixtures.

The fourth part of the work was developed by analysing the biodegradability of mixtures of biodiesel and fossil derived fuels, such as crude oil diesel fuel and gasoline. The CO_2 evolution test was used to analyse the biodegradation behaviour of the mixtures. In all the cases cometabolic biodegradation was observed demonstrating that biodiesel enhances the degradation of the three fossil derived fuels analysed. The physical properties of the mixtures were also analysed.

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RESUMEN

El biodiesel es un combustible de origen renovable que se obtiene a partir de aceites vegetales y grasas animales. Sus propiedades son similares a las del gasoil, por lo que puede utilizarse como combustible en motores diesel, tanto en estado puro como en mezclas con gasoil. El biodiesel se obtiene mediante la reacción de transesterificación, en la que los triglicéridos que componen los aceites y grasas, reaccionan con un alcohol de cadena corta, en presencia de un catalizador. Como resultado de la reacción se obtienen dos fases líquidas, biodiesel en la fase superior y glicerol en la fase inferior. Los alcoholes utilizados en la reacción son generalmente metanol y etanol. Los catalizadores más utilizados son los hidróxidos de sodio y de potasio, aunque también se ha investigado el uso de metóxidos, ácidos inorgánicos y lipasas. Los aceites más utilizados en la producción de biodiesel son los de soja, colza, girasol y palma, aunque existen numerosas alternativas como los aceites de fritura reciclados, las grasas animales, etc.

En este trabajo se utilizó el aceite de Cynara cardunculus, una planta de origen silvestre, de la familia de la alcachofa, también conocida como cardo silvestre o cardo de Castilla. Esta planta se encuentra perfectamente adaptada al clima mediterráneo, soportando condiciones extremas de temperatura y humedad. Su cultivo puede alcanzar una producción de 30-35 toneladas por hectárea al año, de las cuales se obtienen 2 toneladas de semilla. Dicha semilla puede llegar a contener hasta un 25 % de aceite, el cual presenta una composición similar al aceite de girasol. De este modo, el Cynara cardunculus puede representar un cultivo alternativo para la producción de biodiesel, pudiéndose cultivar en tierras abandonadas o no aptas para otro tipo de cultivos.

El presente trabajo se encuentra dividido en cuatro partes principales:

➢ Una introducción general

➢ El estudio de la producción de biodiesel a partir de aceite crudo de Cynara cardunculus

➢ La caracterización de las mezclas de biodiesel con gasoil comercial

➢ El estudio de la biodegradación de mezclas de biodiesel con algunos combustibles de origen fósil

En al primera parte de este trabajo se explica la necesidad de la utilización de energías renovables, de los biocombustibles en particular y entre ellos el biodiesel. Se detalla además la historia del desarrollo de los motores diesel, la historia del biodiesel, su situación actual, su comparación con el gasoil convencional y las características principales de su producción.

En la segunda parte, se optimizaron las condiciones de reacción para la transesterificación de aceite de Cynara cardunculus sin refinar. Las mejores condiciones de operación se obtuvieron para la reacción realizada a 60ºC, utilizando metanol en una relación molar de 6:1 con respecto al aceite, y un 1% de NaOH como catalizador (% en peso de aceite).

El producto obtenido fue caracterizado, determinando los cambios en la viscosidad, y el contenido en metilésteres, monoglicéridos, diglicéridos, triglicéridos y glicerol. El índice de acidez del aceite original resultó ser de 11.8 mgKOH/g, un valor bastante mayor a lo recomendado para la transesterificación mediante catálisis alcalina (1-2 mgKOH/g). A pesar de que los resultados fueron aceptables para algunas condiciones de reacción, para la mayoría de las condiciones experimentadas la reacción condujo a la formación de gel y jabón, dificultando la separación de las fases de glicerol y metilésteres. Estos resultados reflejan la necesidad de realizar una etapa de preesterificación de los ácidos grasos libres, de modo de reducir el índice de acidez.

La etapa de preesterificación fue optimizada utilizando diferentes temperaturas y tiempos finales de reacción, además de variar las concentraciones de catalizador (ácido sulfúrico) y metanol. Las mejores condiciones de operación se obtuvieron para la reacción realizada a 60ºC, utilizando metanol en una relación molar de 6:1 con respecto al aceite, y un 0.5% de H_2SO_4 como catalizador (% en peso de aceite).

Finalmente, el aceite fue pre-tratado bajo las condiciones óptimas de preesterificación, reduciendo su índice de acidez a valores inferiores a 1mgKOH/g. Se realizó además una etapa previa de degomado del aceite para eliminar los fosfolípidos presentes.

La reacción de transesterificación del aceite pre-tratado fue optimizada y los resultados comparados con los de la reacción del aceite crudo. El contenido en metilésteres fue superior al utilizar aceite pre-tratado, y las fases de metilésteres y glicerol se separaron con facilidad en la mayoría de los casos, demostrando la utilidad de las etapas de degomado y preesterificación.

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La tercera parte de este trabajo consiste en la caracterización de las mezclas de biodiesel con gasoil comercial. Actualmente existe en Europa una normativa específica para el biodiesel (EN 14214) y otra específica para el gasoil (EN 590), detallándose en ambas los requerimientos y los métodos de ensayo para diferentes propiedades. Sin embargo, las mezclas de ambos combustibles deben cumplir con la normativa vigente para el gasoil, a pesar de que en algunos casos el biodiesel no cumple con la misma. Para determinar el comportamiento de las mezclas, se prepararon mezclas de ambos combustibles en diferentes proporciones y se analizaron sus propiedades basándose en la normativa europea vigente para el gasoil (EN 590). Para cada una de las propiedades especificadas se realizó la evaluación del comportamiento de las mezclas, explicando los métodos de análisis y la importancia de cada propiedad. Para algunas propiedades fue necesaria la realización de experimentos (densidad, viscosidad, destilación, índice de cetano, punto de inflamación, punto de obstrucción de filtro frío, estabilidad a la oxidación). Las restantes propiedades fueron evaluadas mediante balances de materia. Además, se determinaron las correlaciones apropiadas para calcular las propiedades de las mezclas conociendo las propiedades de los componentes puros y el porcentaje de biodiesel en la mezcla. Dichas correlaciones permiten además, determinar si todas las proporciones de mezcla cumplirán la normativa establecida para el gasoil, o si existe un máximo de biodiesel en la mezcla a partir del cual dicha mezcla dejará de cumplir la normativa. Se determinó que algunas propiedades (la densidad, la viscosidad, la destilación, la estabilidad a la oxidación, el punto de obstrucción de filtro frío, el contenido de agua y el contenido de cenizas) pueden limitar la cantidad de biodiesel permitida en la mezcla para que esta cumpla con la normativa vigente para el gasoil. En algunos casos el no cumplimiento de la normativa implica la diferencia en los limites requeridos para el gasoil (EN 590) y para el biodiesel (EN 14214), sin que esto represente un problema en el funcionamiento de la mezcla en el motor. En estos casos, se debe establecer una normativa específica para las mezclas que se ajuste a sus características. En otros casos, los métodos estándar aplicados al gasoil resultaron poco precisos o incorrectos al aplicarlos al biodiesel y a las mezclas. Esto se puede solucionar mediante el desarrollo de métodos de análisis específicos para las mezclas. Otras propiedades, como la estabilidad a la oxidación y el punto de obstrucción de filtro frío, pueden representar inconvenientes reales en el funcionamiento de las mezclas en el motor y por tanto son las que determinan el máximo de biodiesel permitido en la mezcla.

La cuarta parte de este trabajo consistió en el análisis de la evolución de la biodegradación de las mezclas de biodiesel con combustibles de origen fósil (petróleo crudo, gasoil y

gasolina). Se realizaron mezclas de biodiesel con dichos combustibles en diferentes proporciones, determinando en cada caso las propiedades físicas de las mezclas (densidad y viscosidad). La biodegradación se determinó mediante el método de evolución de CO_2. En todos los casos, los efectos sinérgicos fueron positivos indicando la presencia de cometabolismo. Se demostró por tanto, que el biodiesel incrementa la biodegradabilidad de los tres combustibles fósiles examinados, permitiendo de este modo su utilización en la el tratamiento de derrames de los mismos. Se proponen además algunas posibilidades de utilización.

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ABBREVIATIONS

A	Arrhenius pre-exponential factor
AC	Ash content
B	Alkaline catalyst
B	Biodegradability
BD	Biodiesel
BTEX	Benzene – Toluene – Ethylbenzene – Xylene
C	Concentration
CF	Control flask
CFPP	Cold Filter Plugging Point
CI	Cetane index
CR	Carbon residue
Ct	Theoretical carbon
DF	Diesel Fuel
DG	Diglycerides
Ea	Activation energy
EU	European Union
FAME	Fatty Acid Methyl Esters
FF	Fossil Fuel
FFA	Free Fatty Acids
GC	Gas Chromatography
GL	Glycerol
HPLC	High Precision Liquid Chromatography
IS	Internal Standard
k	Specific rate constant
K	Equilibrium rate constant
LC	Lineal combination
m	Mass
M	Molecular weight
MeOH	Methanol
MG	Monoglycerides
P	Percentage
PAH	Polycyclic Aromatic Hydrocarbons

R	Gas constant (8.314 J/mol.K)
R, R', R'', R'''	Carbon chain
ROH	Alcohol
S	Substrate
SC	Sulphur content
SF	Synergic factor
T	Temperature
TC	Total contamination
TF	Test flask
TG	Triglycerides
WC	Water content
X	Mass fraction
μ	Viscosity
δ	Density

1. INTRODUCTION

1.1. GENERAL BACKGROUND

1.1.1. ENVIRONMENTAL ISSUES

During the generation, transport and consumption of conventional forms of energy, the environment is affected in several ways, including the emissions of greenhouse gases and other contaminants. This is the origin of mayor environmental problems like the climate change and the acid rain. Some solutions to this problem are the treatment of combustion gases, the stimulation of energy efficient technologies, and a major use of renewable forms of energy *(United Nations, 1997)*.

According to the Kyoto Protocol, the emissions of greenhouse gases should be reduced by at least 5 % taking 1990 levels as reference, during the period 2008-2012 *(United Nations, 1997)*. To meet this goal, the European Union (EU) has agreed to attain by 2010 a minimum penetration of 12 % of renewable energy sources, being biomass the main contribution with 7 % of the total consumption *(European Commission, 1997)*. This includes the use of solid, liquid or gaseous forms of biomass. In the case of liquid biomass (or biofuels), the objectives of the EU for 2020 include increasing their consumption with a target of 20 % for all fuel substitutes *(European Commission, 2000)*.

1.1.2. THE PEAK OIL

During the last years, the price of petroleum has been climbing while the global energy demand is rising 1-2 % each year. New exploitation discoveries require every time more investment, being also more difficult to exploit and less prolific. The world is nowadays consuming two barrels of petroleum for every one found. The moment when petroleum consume will be higher than the petroleum available is usually known as the peak oil, and it has been estimated that it will occur at any time between 2007 and 2030. However, there is no agreement on actual available resources and reserves *(M. Marzo, 2006)*.

1.1.3. RENEWABLE ENERGY FOR THE TRANSPORT SECTOR

At present, petroleum and its derivatives represent almost 35 % of the total primary energy supply in the world. From them, almost 60 % is employed in the transport sector. On the other hand, renewable energies still represent a small proportion when compared to fossil fuels use *(M. Marzo, 2006)*. Many alternative forms of renewable energy, such as wind, solar and geothermal, are acquiring importance in the gradual replacement of fossil fuels. Nevertheless, these forms of energy are useful for electricity generation, but present technology is not practical for their use in transportation. Some alternatives such as the use of compressed natural gas (CNG) or hydrogen powered fuel cells, are being considered as possible solutions for the transportation sector. Though CNG is being used in several countries with low prices and clean burning, it is still a fossil fuel and is suffering price increases and production decreases as well as petroleum is. In addition, it requires expensive equipments and vehicle modifications. On the other hand, the technology for hydrogen production and distribution is still under development and will take more time to be available for transport use *(Pahl, 2005)*.

In the meantime, liquid biofuels from renewable sources are acquiring importance as they can be used without the need of engine modifications and can represent an alternative to at least a portion of the petroleum fuels market *(Pahl, 2005)*. There are different types of biofuels, from which the most important are bioethanol (which is used to partially or totally replace gasoline), and biodiesel.

This thesis is focused on biodiesel, a clean renewable fuel, obtained from organic feedstocks as vegetable oils or animal fats.

Biodiesel (fatty acid alkyl esters) is produced through the transesterification of the oil or fat, with a short chain alcohol in the presence of a catalyst (usually NaOH or KOH). The physico-chemical properties of biodiesel are similar to those of fossil diesel fuel (Table 1.1), so it can be used to partially or totally replace diesel for the use in compression ignition engines. Biodiesel has many environmental advantages as it considerably reduces emissions of contaminants, it is readily biodegradable and non-toxic.

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Table 1.1. Comparison of some physico-chemical properties of vegetable oils, biodiesel and fossil diesel fuel.

Property	Vegetable Oil	Biodiesel	Diesel
Density (Kg/m^3)	916-924	880	835
Viscosity (cp-20ºC)	65.8-77.8	7.5-8.0	5.1
Viscosity (cp-50ºC)	25.7-34.9	3.8-4.2	2.6
Cetane Number	33-51	45-56	> 45
Carbon residue %	0.25-0.42	0.02-0.05	0.15
Sulphur %	0.0001-0.01	0.002-0.01	0.29

* Source: Biofuels – European Commission

1.1.4.   BIODIESEL HISTORY

Rudolf Diesel was born in Paris in 1858, though his family had a German origin. During the Franco-Prussian War he went to study in Augsburg, Germany, where he assisted to a technical school and to a mechanical engineering program. Then he studied thermodynamics at the Munich Institute of Technology where he began to develop his idea on a *heat engine*, that would not need a spark plug to create combustion. The main source of industrial power at that time was the steam engine, but it was extremely expensive and had less than a 10 % efficiency. While he worked for several companies and acquired practical mechanical experience, he began experiments with his engines. In 1892 he published a paper called "*Theory and Construction of a Rational Heat Engine to Replace the Steam Engine and Contemporary Combustion Engine*" where he described his invention as a "compression ignition engine". This engine could burn virtually any fuel as it was ignited not by a spark but by the extremely high temperature caused by the high compression of the air before the injection of fuel into the cylinder *(Pahl, 2005)*.

The first prototype was presented in 1893 in Augsburg and was initially fuelled by kerosene and gasoline, though it did not generate enough power. He continued refining the model and trying different fuels, such as lighting gas and heavy oils. He patented the third prototype and four of his engines were shown at the Munich Power and Machinery Exhibition in 1898. A smaller version of Diesel's engine was shown at the Paris Exhibition in 1900, and this was the first time it run on peanut oil *(Pahl, 2005)*.
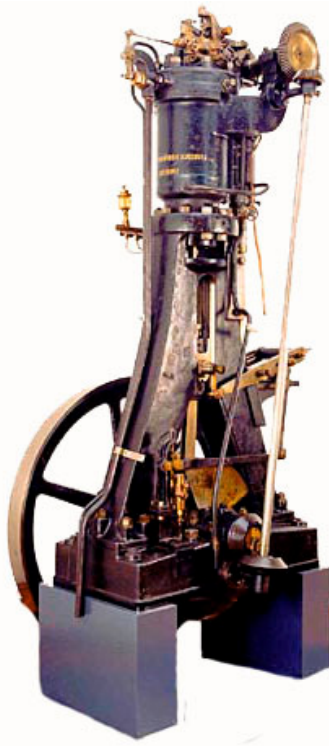
Figure 1.1. Diesel's engine prototype

In the following years, Diesel continued testing vegetable oils to power his engine. As his desire was to create an engine that could be operated almost anywhere on almost any fuel, he focused on the use of renewable fuels such as castor oil, palm oil, lard and other seed oils *(Pahl, 2005)*.

Because of their size and weight, the first diesel engines were mostly used in factories, electrical generation plants, and ships. Automotive industry started using diesel engines after 1924, with the development of smaller, lighter engines with self contained direct injection pumps. The original Diesel's idea to use his engines with a variety of fuels, was displaced after his death in 1913, by the use of cheap and plentiful petroleum based kerosene. The diesel fuel as it is known today was developed in the twentieth century as diesel engines came into wider use. The number of diesel engines that were manufactured to run on diesel fuel grew exponentially *(Pahl, 2005)*.

Experiments with vegetable oils continued from 1920 to 1940 in the European nations that had tropical colonies. Alkyl esters use in diesel engines was first reported in a Belgian patent on the use of palm oil ethyl esters, in 1937. Vegetable oils were used as emergency fuels in South American and Asian countries, during World War II, but their use was interrupted after the war, as cheap petroleum based fuels were available again *(Pahl, 2005)*.

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For many years the availability of inexpensive petroleum fuels provided little incentives for experimenting with alternative, renewable fuels for diesel engines. However since the oil crisis in 1970's research interest has expanded in the area of alternative fuels *(Canakci, 2001)*.

Research on vegetable oils use for diesel engines, started again after the 1970's energy crisis and was mainly conducted in Austria, South Africa and the United States *(Pahl, 2005)*. Different types of oils such as palm, soybean, sunflower, peanut and olive oils were used for diesel engines. However vegetable oils present different fuel properties when compared to diesel fuel, as they have higher density, viscosity and flash point, and lower cetane number and heating values. The direct use of vegetable oils presented engine problems after long time use due to the oil characteristics and cold weather conditions. They present injection problems, poor fuel atomization, incomplete combustion, deposits formation, carbonization of injector tips, ring sticking, lubricating oil dilution and degradation *(Srivastava, 2000)*. Thus, in 1973, professors Manfred Wörgetter and Josef Pernkopf from BLT in Wieselburg and professors Martin Mittelbach and Hans Junek from the University of Graz, focused their research in adapting the oils to the diesel engines. They found the transesterification reaction to be useful for the modification of vegetable oils, obtaining fatty acid alkyl esters (later known as biodiesel) and glycerol. They also patented the first method to produce cheap biodiesel with a low-temperature and low-pressure process. They constructed the first biodiesel pilot plant, that produced around 500 tons rapeseed oil biodiesel annually, and developed the first biodiesel standard (ON C 1190). Professor Mittelbach was also the first to use biodiesel in his own car *(Pahl, 2005)*.

1.1.5.   CURRENT BIODIESEL STATUS

Biodiesel industry is beginning to experience a dramatic growth in many parts of the world. While fossil fuels were formed over millions of years, biodiesel can be locally created in just a few months from renewable sources *(Pahl, 2005)*.

Nowadays, more than 25 countries are using biodiesel pure or in mixtures with diesel. It is usually obtained from soybean, rapeseed or sunflower oils, but some alternative feedstocks are coconut, palm, algae, animal fats and recycled frying used oils. In Europe, the main biodiesel producers are Germany, France, Italy, Austria and Denmark (Table 1.2)

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Table 1.2. Biodiesel production in Europe during 2005, and estimate production for 2006

Country	2005 biodiesel production (in thousands of tons)	Estimate production for 2006 (in thousands of tons)
Germany	1669	2681
France	492	775
Italy	396	857
Czech Republic	133	203
Poland	100	150
Austria	85	134
Slovakia	78	89
Spain	73	224
Denmark	71	81
UK	51	445
Slovenia	8	17
Others	28	671
Total	**3184**	**6069**

* 2005 production has increased by 65% when compared to 2004 production
* Source: European Biodiesel Board

The production of biodiesel in Spain has increased in the last years with some plants already working in Montmeló, Reus and Alava (18000, 50000 and 55000 tons/year respectively), and some other plants in project. There are also several fuel stations providing mixtures of biodiesel and diesel, in Catalunya and Aragon.

Figure1.2. Biodiesel dispenser in a Shell fuel station in Tarragona, Spain.

Biodiesel is receiving increased attention as an alternative, non-toxic, biodegradable and renewable diesel fuel. It has a higher cetane number than diesel fuel, no aromatic compounds, almost no sulphur and contains 10-11 % oxygen by weight, thus reducing emissions of carbon monoxide, hydrocarbon and particulate matter *(Canakci, 2001)* . For up to twenty years, biodiesel has been in commercial use in many countries, including Austria, Czech Republic, Germany, France, Italy, Spain and USA *(Mittelbach, 2004)*.

1.1.6.   BIODIESEL PRODUCTION

Biodiesel is obtained from organic feedstocks such as vegetable oils or animal fats. It is mainly produced through the transesterification of the oil or fat, with a short chain alcohol in the presence of a catalyst.

Oils and fats are composed primarily by triglycerides, that consist of a glycerine backbone with fatty acid radicals attached in place of the hydroxyls. Transesterification is a reaction between the triglycerides and short chain alcohols, that gives free glycerol and the fatty acid esters of the respective alcohol, known as biodiesel.

In the transesterification reaction (figures 1.3 and 1.4), one mole of triglyceride reacts with three moles of alcohol to form one mole of glycerol and three moles of the respective fatty acid alkyl esters, in a sequence of three reversible reactions where the triglycerides are converted to diglycerides, monoglycerides and finally glycerol. The yield of the reaction is improved by adding an excess of alcohol and a catalyst. As glycerol and the esters are hardly miscible, they form two phases that can be separated *(Mittelbach, 2004)*.

$$
\begin{array}{ccccccc}
H_2C-OCOR' & & & & ROCOR' & & H_2C-OH \\
| & & & & + & & | \\
HC-OCOR'' & + & 3\,ROH & \overset{catalyst}{\rightleftharpoons} & ROCOR'' & + & HC-OH \\
| & & & & + & & | \\
H_2C-OCOR''' & & & & ROCOR''' & & H_2C-OH \\
\textbf{\textit{Triglyceride}} & & \textbf{\textit{Alcohol}} & & \textbf{\textit{Alkyl esters}} & & \textbf{\textit{Glycerol}}
\end{array}
$$
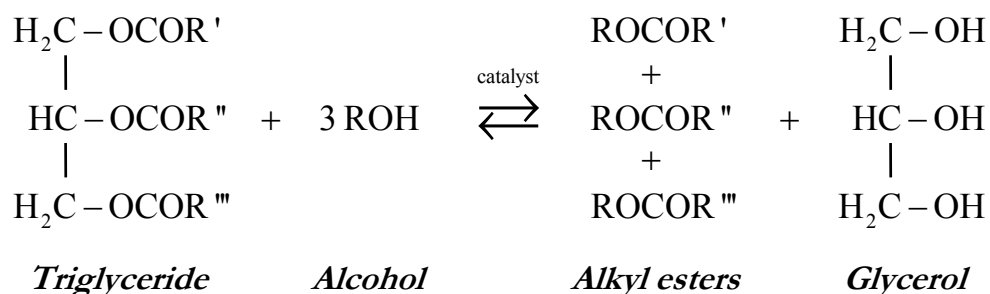
Figure 1.3. Overall transesterification reaction

$$
\begin{array}{c}
H_2C-OCOR' \\
| \\
HC-OCOR'' \\
| \\
H_2C-OCOR'''
\end{array}
+ CH_3OH
\underset{k_2}{\overset{k_1}{\rightleftharpoons}}
H_3C-OCOR'
+
\begin{array}{c}
H_2C-OH \\
| \\
HC-OCOR'' \\
| \\
H_2C-OCOR'''
\end{array}
$$

Triglyceride   **Methanol**   **Methyl ester**   **Diglyceride**

$$
\begin{array}{c}
H_2C-OH \\
| \\
HC-OCOR'' \\
| \\
H_2C-OCOR'''
\end{array}
+ CH_3OH
\underset{k_4}{\overset{k_3}{\rightleftharpoons}}
H_3C-OCOR''
+
\begin{array}{c}
H_2C-OH \\
| \\
HC-OH \\
| \\
H_2C-OCOR'''
\end{array}
$$

Diglyceride   **Methanol**   **Methyl ester**   **Monoglyceride**

$$
\begin{array}{c}
H_2C-OH \\
| \\
HC-OH \\
| \\
H_2C-OCOR'''
\end{array}
+ CH_3OH
\underset{k_6}{\overset{k_5}{\rightleftharpoons}}
H_3C-OCOR'''
+
\begin{array}{c}
H_2C-OH \\
| \\
HC-OH \\
| \\
H_2C-OH
\end{array}
$$

Monoglyceride   **Methanol**   **Methyl ester**   **Glycerol**

Figure 1.4. Transesterification reaction steps

The transesterification reaction depends mainly on the nature and concentration of catalyst, the molar ratio of alcohol to oil, the temperature, pressure and reaction time, and the content of water and free fatty acids (FFA) *(Sendzikiene, 2004)*.

Traditional raw materials for biodiesel production are the oils of rapeseed, sunflower, soybean and palm, as these crops have the highest current worldwide production. The choice of raw material in a specific region mainly depends on the respective climatic conditions *(Mittelbach, 2004)*. As the main drawback of biodiesel is price, different raw

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materials have to be tested in order to reduce production costs and reach final product prices that can compete with fossil diesel fuel. Lower cost feedstocks, such as high FFA oils, are needed *(Canakci, 2001)*. Some alternative edible oils that are being tested are *Brassica carinata* (Ethiopian mustard) and *Cynara cardunculus* in the Mediterranean countries, *Camelina sativa* (gold of pleasure) and *Linum usitatissimum* (linseed) in Central Europe, *Cyperus esculentus* (tigernut) in Africa, *Elaeis guineensis* (palm kernel) and *Cocos nucifera* (coconut) in South Asia and *Brassica nigra* and *Sinapis alba* (mustard oils) in USA. Some non-edible oils are also good candidates for biodiesel production as they contain substances which make them unsuitable for human consumption but considerably cheaper than edible oils. Some examples are *Ricinus communis* (castor oil) in Brazil, *Jatropha curcas* (physic nut) in Nicaragua and some oils of Indian origin like *Shorea robusta* (sal), *Madhuca latifolia* (mahua), *Antelaea azadirachta* (neem) and *Pongamia glabra* (karanja) oils. Animal fats and recycled oils are also popular raw materials for biodiesel production as they are inexpensive and represent an additional benefit for the environment with the use of waste material *(Mittelbach, 2004)*.

There are different types of catalysts employed in the transesterification reaction, which are mainly divided into alkaline, acid and enzymatic catalysts.

Alkaline catalysts, such as NaOH and KOH are the most commonly used for biodiesel production. Figure 1.5 explains the alkaline catalysed transesterification reaction mechanism for triglycerides. Mono and diglycerides react by the same mechanism *(Schuchardt, 1998)*. When compared with acid or other type of catalysts, basic ones show a high conversion under mild conditions and in short reaction times. In addition, they are less corrosive to industrial equipment and require smaller volumes of alcohol, thus reducing reactor sizes. Alkaline metal alkoxides are the most active catalysts as they give high yields in short times but they require the absence of water. Alkaline hydroxides are less active but cheaper, though they are the most widely used in industrial processes *(Ma, 1999)*.

Acid catalysts also give high yields, but the reaction is slow and requires higher temperatures. Figure 1.6 explains the acid catalysed transesterification mechanism *(Schuchardt, 1998)*. The most common acids used are phosphoric, hydrochloric, sulfonic and sulfuric acids. Acid catalysts are recommended for oils having a high FFA content, as they also catalyse the esterification reaction of the FFA. They are also employed in pre-treatment steps, to esterify the FFA prior to basic catalysed reaction. Nevertheless acid catalysis is also affected by the presence of water, that inhibits the reaction.

$$ROH \quad + \quad B \quad \rightleftharpoons \quad RO^- \quad + \quad BH^+$$
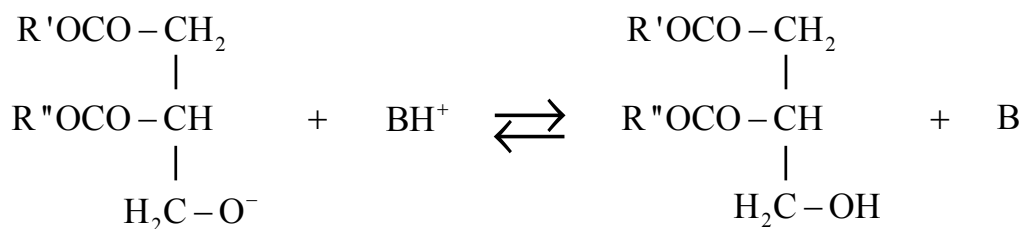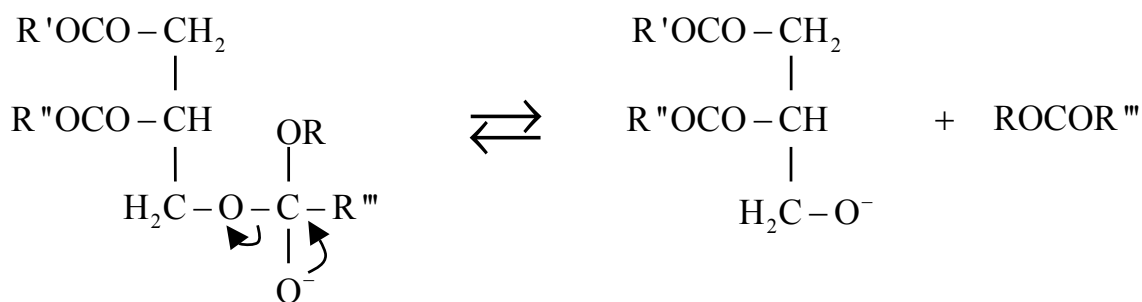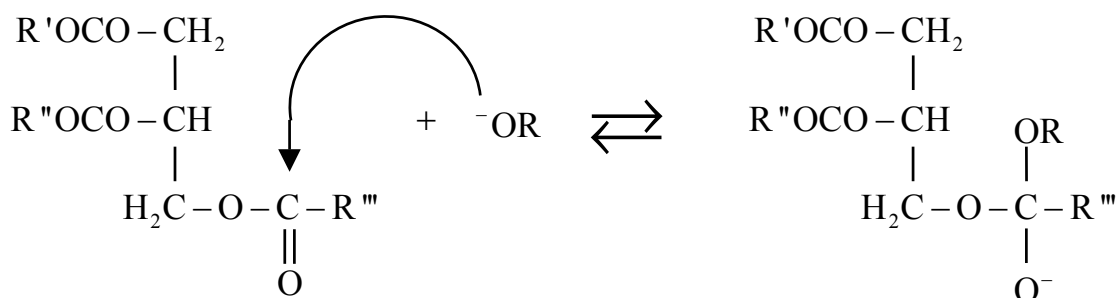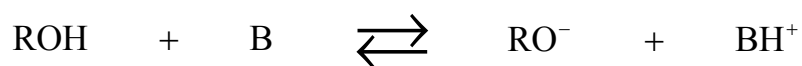
ROH : alcohol

B : alkaline catalyst

R, R′, R′′ and R′′′ : carbon chains

Figure 1.5. Mechanism for the alkaline catalysed transesterification

ROH : alcohol

R, R′, R′′ and R′′′ : carbon chains
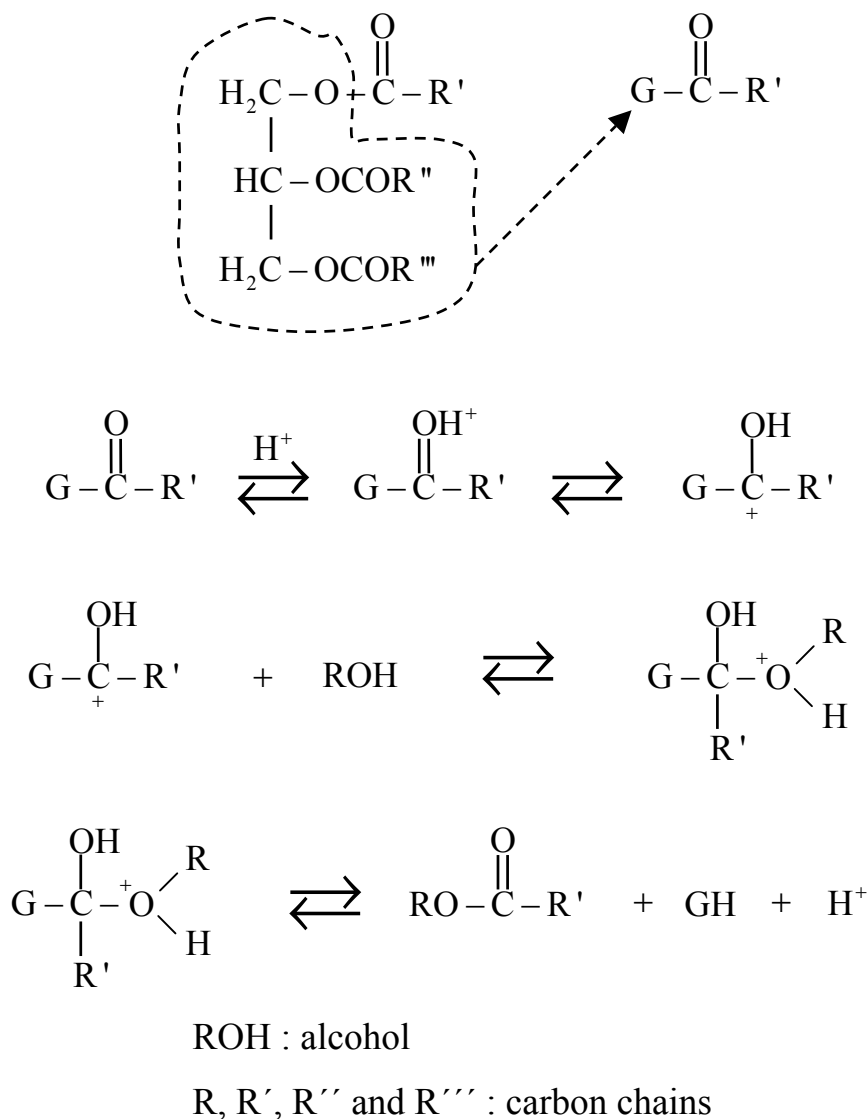
Figure 1.6. Mechanism for the acid catalysed transesterification

Enzymatic catalysts give high yields in the transesterification of vegetable oils despite the presence of water and FFA. They also facilitate glycerol recovery. However, enzymatic catalysts are significantly more expensive than basic or acid ones *(Fukuda, 2001)*.

The alcohols used in the reaction are short chain alcohols, mainly methanol and ethanol, but also propanol and butanol are used. Methanol has a the lowest cost, and presents some chemical and physical advantages over the rest of alcohols, as it quickly reacts with triglycerides and easily dissolves NaOH *(Ma, 1999)*. The methanol to oil molar ratio employed is usually 6:1 (twice the stoichiometric amount), when working with basic catalysts. The excess methanol can be recovered and reused after the reaction. Ethanol also presents high reaction yields, but dry ethanol is more expensive than ethanol and also more

difficult recover once it has been in contact with water. Longer chain alcohols are more expensive and require higher molar ratios and higher temperatures. They are also unable to dissolve NaOH and KOH and thus they usually employed for acid catalysis.

1.1.7. BIODIESEL VS DIESEL FUEL

There are many reasons to encourage the use of biodiesel and to increase the diesel fuel replacement. Some of them are technical, others are related to environmental aspects, but among them the most important are that:

➢ It is obtained from renewable sources

➢ It can be locally produced, thus reducing the need for foreign imports of fossil fuels and creating jobs

➢ It is biodegradable and non-toxic

➢ It reduces most exhaust emissions

➢ Its handling and storage is safer due to he high flash point

➢ It is an excellent lubricant

However, there are still some limitations that have to be solved:

➢ Its final price highly depends on the original oil, making it usually more expensive than diesel fuel

➢ It presents a low stability when it is exposed to air

➢ Its cold flow properties can limit its use in cold weather

The raw material used in the production of biodiesel acquires great importance, not only because of its influence on the final biodiesel properties but also because of its cost.

1.2.   OBJECTIVES OF THIS THESIS

The main objective of this work is to optimise the reaction conditions for the production of biodiesel from the oil of *Cynara cardunculus*, an alternative oil crop typical from the Mediterranean area, and to evaluate some specific applications of biodiesel. Specific aims related to the main objectives are:

➢ To optimise the reaction conditions for the production of biodiesel from unrefined *Cynara cardunculus* oil

➢ To evaluate and optimise the use of pre-treatment steps before biodiesel production

➢ To compare the properties of the biodiesel obtained from both unrefined and pre-treated *Cynara cardunculus* oil

➢ To propose and evaluate a kinetic model for the preesterification and transesterification reactions

➢ To set up analytical methods based on European normative in order to characterise the product obtained

➢ To characterise the mixtures of biodiesel with commercial diesel fuel, according to diesel fuel standard requirements

➢ To evaluate the possible applications of biodiesel to cometabolically degrade fossil derived fuels.

1.3. THESIS OUTLINE

This thesis is divided into three main parts, each of them reflected in a separated chapter.

Chapter 2 describes the procedure to obtain biodiesel from unrefined *Cynara cardunculus* oil, both with and without any pre-treatment steps, the improvement of the pre-treatment steps and the reaction kinetics. This chapter is divided into three main parts, the reaction behaviour when the original unrefined oil is used, the optimisation of the preesterification steps, and the reaction behaviour when pre-treated oil is used.

Chapter 3 presents a comparison between the standard analytical methods and requirements for both diesel fuel and biodiesel, and the behaviour of biodiesel-diesel fuel mixtures when they are analysed under diesel fuel requirements.

Chapter 4 presents the biodegradation behaviour of biodiesel and its mixtures with a heavy crude oil and some fossil derived fuels (diesel fuel and gasoline). It also describes the properties of the mixtures and some possible applications in the case of an accidental spill. Some of these results are published in *Biomass and Bioenergy*.

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2.   BIODIESEL PRODUCTION FROM CYNARA CARDUNCULUS OIL

"Soy como el cardo salvaje

nací en el campo no más

nunca doy un paso atrás

y sé aguantar los rigores

Pa' los amigos las flores

y espinas pa' los demás".

"Soy como el cardo salvaje

arisco pa' la ciudad

me gusta la soledad

y la quietud del paisaje......"

Esperón, s/f

2.1. INTRODUCTION

Cynara cardunculus is a non-domesticated robust perennial plant, characterised by its rosette of large spiny leaves, branched flowering stems and blue-violet flowers. As well as artichoke, sunflower and safflower, it belongs to the family of Asteraceae, tribe Cynareae and is native from the Mediterranean basin, where it colonises dry and undisturbed areas. It is allogamous and is propagated by seeds, most of which are shed close to the parent plant. Flowers are produced from March to July at different stages of plant maturity and usually by 2-year-old plants *(Portis 2005)*.

Figure 2.1. Wild *Cynara cardunculus* plant

The growth cycle of the plant is the following *(Gominho 2001, Portis 2005, Curt 2002)*:

➢ During the first rains after summer: seeds germination (although germination may occur all year around under favourable conditions)

➢ During autumn: emergence period

➢ During winter: the leaf rosette appears

➢ During spring: achene maturity period

> ➢ During summer: the aboveground part of the plant dries off, and it can be harvested, while the underground part starts a new cycle.

The maximum production reaches 30-35 tons/ha per year. However the average production is of about 20 tons/ha per year. The stalks constitute the 40 % of the plant, with a 14.6 % of extractives, 17 % of lignin, 53 % of polysaccharides (mainly cellulose and xylan) and 18 % of moisture. The leaves and the capitula constitute the 25 and 35 % of the plant respectively *(Gominho 2001)*. The capitula, or flower head, is a cluster of flowers so arranged that the whole gives the effect of a single flower and contains almost 6 % of seeds.

Cynara cardunculus has been only used as a forage for many years, but new applications have been discovered recently. If properly prepared, immature flowers, petioles and roots are edible. This plant has been used in regional food dishes as soups, salads and as a coagulant for cheese making *(Gominho 2001, Portis 2005)*. The seeds can be used to obtain edible oil, being a promising source of it, both with respect to quantity and quality, and the residual cake after extraction is usable as a component of animal feed *(Portis 2005)*. The anatomical characterisation of the stalks indicate that they are appropriate for paper sheet forming and have good strength properties *(Benjelloun 1997, Quilho 2004)*.

Cynara cardunculus species is scattered in Europe, North Africa, Madeira and Canary Islands. In the Iberian Peninsula it is mostly found in the South of Portugal and Andalucia in Spain *(Gominho 2001, Curt 2002)*. In Italy, populations of wild cardoon can be found in different areas within the islands of Sicily and Sardinia *(Portis 2005)*. Naturalised cultivars can be found in California, Mexico, Australia and Southern South America, where it is known as "*cardo de Castilla*" (Castilian cardoon) *(Encinar 1999)*. In Argentine, the plant was introduced by the Spaniards during the XVIII century, probably by accident, mixed with other crop seeds.

Cynara cardunculus grows in dense areas across a variety of habitats, including mesic riparian sites as well as xeric rocky slopes, and covering locations with different climatic and environmental conditions. Its deep root system allows the plant to extract water and nutrients from very deep soil zones. The roots can attain almost seven meters deep, reaching underground rainwater permeates. Thus, they can make good use of lixiviated fertilisers from previous crops. The colonisation of new habitats and the gene flow

between populations occurs primarily via seed dispersal by birds and wind. A high genetic variation is present within populations *(Portis 2005)*.

For a large scale cultivation of *Cynara cardunculus*, two successive stages must be developed: the implantation and the production periods. The implantation period occurs during the first year, with a low biomass production obtained while the root system is developed. This period implies the fertilisation, plowing, harrowing and sowing of the land followed by herbicide and insecticide treatments, with an estimated cost of 350 euro/ha. The production period covers the productive life of the crop, that starts in the second year, and lasts almost ten years. It implies the fertiliser restoring, herbicide and insecticide treatments and harvesting, with an estimated cost of 630 euro/ha *(Gominho 2001)*.

Some authors *(Foti 1999, Maccarone 1999)* studied the potential of *Cynara cardunculus* for biomass production, the seed yield, and the composition of the grain and the oil extracted. In a 3-year experiment located in Italy, they obtained more than 2 tons per ha/year grain and 30 tons per ha/year of dry biomass. The grain contained 20.1 % crude protein, 24.4 % oil, 18.5 % crude fiber and 4.1 % ash.

Benjelloun et al (1997) obtained 25 % oil in dry matter after soxhlet extraction with hexane from *Cynara cardunculus* seeds. The physicochemical properties of the oil were comparable to sunflower oil. The oil composition obtained was 60.9 % linoleic, 23.6 % oleic, 12.1 % palmitic and 3.4 % stearic acid *(Benjelloun 1997)*.

A perennial cultivation system was used to study the potential of *Cynara cardunculus* as an oil crop, comparing the seed oil content and the fatty acid oil composition for different plant population, crop year and experiment location. Eighteen *Cynara cardunculus* populations were studied in a 3-year experiment located in Madrid, and a standard population grown from 1 to 10 years in different locations of Spain and Italy. The oil content obtained was 20-32 % in dry matter, depending on the seed size and population. The age of the crop and the location had almost no effect on oil content. The average composition of the oil was similar to sunflower oil *(Curt 2002)*.

The results reported above show the possibility of obtaining from 400 to 650 kg per ha/year of *Cynara cardunculus* oil, as a result of the large scale cultivation of the plant. These oil yields are thus comparable to that obtained from sunflower or soybean (800 and 375 kg per ha/year respectively, *Mittelbach 2004*).

As the plant is well adapted to environments characterised by extreme light, temperature and atmospheric conditions, its cultivation could be extended to the unfavourable edaphic and climatic conditions of many inland hilly areas of the Mediterranean basin *(Portis 2005)*.

Figure 2.2. The flower of *Cynara cardunculus*

Figure 2.3. The seed of *Cynara cardunculus*

2.2. ONE STEP TRANSESTERIFICATION REACTION

2.2.1. INTRODUCTION

Other authors *(Encinar 1999, Encinar 2002)* have studied the use of *Cynara cardunculus* oil for the production of biodiesel. They prepared biodiesel via transesterification of refined *Cynara cardunculus* oil, using different alcohols, catalysts and operation conditions.

Encinar et al *(1999)* used methanol at different molar ratios and with different catalysts (NaOH, KOH or $NaOCH_3$) at temperatures ranging from 25 to 60ºC for a reaction time of 120 min. The best yields were obtained for methanol:oil molar ratios higher than 4:1. Methanol ratios lower than 4:1 gave incomplete reaction. Methanol ratios higher than 6:1 hinder glycerol decantation and separation. The three catalysts used gave similar yields, but with different optimal concentrations (0.5 % for NaOH and KOH, and 1 % for $NaOCH_3$). Low catalyst concentration gave low ester yields, as the catalyst is consumed by the FFA neutralisation. High catalyst concentrations compensate FFA neutralisation but can lead to the formation of emulsions and gels, with no glycerol phase separation. The temperature that gave the higher yields was 60ºC, however at room temperature the results are also good (86 % methyl esters). Final conversion was reached in only 10 minutes, with a maximum yield of 93.9 % methyl esters for the best reaction conditions (60ºC, 4:1 metanol to oil ratio, 0.5 % NaOH). The properties of the biodiesel obtained were in all the experiments similar to that of biodiesel prepared from sunflower, rapeseed or soybean oils, also comparable to the properties of a typical diesel fuel.

Encinar et al *(2002)* also used ethanol for biodiesel production with *Cynara cardunculus* oil, obtaining the best yields for ethanol molar ratios between 9:1 and 12:1. For lower molar ratios, the reaction was incomplete, and for higher molar ratios the separation of glycerol was difficult, decreasing the yield of esters. NaOH gave the best yields, however the results for KOH were not much lower. The optimal concentration was 1 % for both catalysts. The temperature that gave the higher yields was 75 ºC, however at room temperature the results are also good (91.6 % ethyl esters). Final conversion was reached in only 10 minutes, with a maximum yield of 93.2 % ethyl esters for the best reaction conditions (75ºC, 12:1 ethanol to oil ratio, 1 % NaOH). The properties of the biodiesel obtained were in all the experiments similar to that of biodiesel prepared from sunflower, rapeseed or soybean oils, also comparable to the properties of a typical diesel fuel. Transesterification with ethanol

increases the cetane number and calorific content of biodiesel, because of the extra carbon atom. It also improves cold weather properties such as cloud and pour points.

The results reported above show the potential of *Cynara cardunculus* cultivation, as an alternative for abandoned cropland and a good candidate as renewable energy source. The biomass and seed production gave promising results, obtaining a grain rich in proteins and an oil comparable to commercial vegetable oils. The biodiesel obtained in the transesterification of the oil can be a good alternative to replace fossil diesel fuel.

2.2.2. *CYNARA CARDUNCULUS* OIL CHARACTERISATION

Unrefined *Cynara cardunculus* oil was obtained from Jesús Fernández (Escuela Técnica Superior de Ingenieros Agrónomos - Universidad Politécnica de Madrid, Spain). The main properties of this oil are shown in table 2.1. All properties were analysed following European standard test methods.

Table 2.1. Properties of unrefined *Cynara cardunculus* oil.

Property	Result	Method
Density (15ºC)	921 kg/m^3	EN ISO 3675
Viscosity (40ºC)	32 mm^2/s	EN ISO 3104
Methyl esters content	0 % (w/w)	EN 14103
Monoglycerides content	0.08 % (w/w)	EN 14105
Diglycerides content	2.78 % (w/w)	EN 14105
Glycerol content	0.0 % (w/w)	EN 14105
Free fatty acids	5.84 % (w/w)	EN ISO 660
Acid value	11.63 mgKOH/g	EN 14104
Iodine value	123.7 gI$_2$/100g	EN ISO 3961
Water content	1178 ppm	EN ISO 12937

For vegetable oils, the FFA content is expressed as the oleic acid percentage. Although the experimental procedure to measure the FFA content in both vegetable oils and biodiesel is similar, the results for biodiesel are expressed as the mg of KOH necessary to neutralise the FFA present in 1g of sample, usually called acid value. In the present work, the acid value is also used for initial oil in order to maintain a coherence with further pre-treated oil and biodiesel properties.

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When the FFA content is lower than 5 %, basic catalyst can still be used for the transesterification reaction, but adding the amount needed to neutralise the FFA. The soap formed during the reaction is removed with glycerol and water washes. When the FFA content is higher than 5 %, the soaps formed during the reaction inhibit the separation of the glycerol and ester phases, and forms emulsions with water washes. In this case, acid catalysed pre-treatment is recommended *(Knothe 2005)*.

As the methyl esters composition of biodiesel coincides with the triglycerides composition of the initial oil, we analysed the methyl esters composition of the transesterified oil, using European standard test method (EN 14103). The results are reported in table 2.2., where they are compared with *Cynara cardunculus* oil composition reported in bibliography.

Table 2.2. Triglycerides composition of *Cynara cardunculus* oil

Fatty acid name	Double bonds	Composition (wt %)	Reported *(Benjelloun 1997/Curt 2002)*
Palmitic	C16:0	11.40	12.1 / 10.66
Palmitoleic	C16:1	-	- / 0.16
Stearic	C18:0	3.60	3.4 / 3.71
Oleic	C18:1	27.00	23.6 / 24.99
Linoleic	C18:2	58.00	60.9 / 59.74
Linolenic	C18:3	-	- / -
Arachidic	C20:0	-	- / 0.36

This composition is similar to that found for sunflower oil (6.0 % palmitic, 3.0 – 5.0 % stearic, 17.0 – 22.0 % oleic, 67.0 – 74.0 % linoleic, 0.6 % behenic acids). The absence of linolenic acid means that the oil has a high oxidation stability. The molecular weight of this oil is 873.2 g/mol, calculated from its composition.

2.2.3. TRANSESTERIFICATION METHODOLOGY AND REACTION CONDITIONS

Unrefined *Cynara cardunculus* oil was transesterified under different reaction conditions. Different short chain alcohols were used, such as methanol, ethanol, iso-propanol and tert-butanol. Reaction with methanol was performed at room temperature (25ºC), 40 and 60ºC. Higher temperatures were used for reaction with ethanol, isopropanol and tert-butanol. Alcohol to oil molar ratios ranging from 6:1 to 15:1 were used. The catalysts used were NaOH and KOH, with concentrations of 0.5, 1.0 and 1.5 % by weight of oil. As

basic catalysts are not soluble in iso-propanol and tert-butanol, H_2SO_4 was used as catalyst in these cases. Reaction time ranged from 10 to 90 minutes for methanol and ethanol, and from 10 to 240 minutes for longer chain alcohols.

Figure 2.4. Transesterification of *Cynara cardunculus* oil. Experimental set-up.

Figure 2.5. Phase separation after transesterification. Biodiesel and glycerol can be seen as the upper and lower phases respectively

All experiments were conducted in 250 ml glass flasks, kept under permanent agitation in a water bath at the desired temperature (Figure 2.4). Reaction kinetics was followed by using 9 reaction flasks for each set of reaction conditions and removing one of the flasks every 10 minutes. Each flask contained 150 ml of *Cynara cardunculus* oil, that was preheated until it reached the experimental temperature. The alcohol and catalyst were premixed at the correspondent proportions, and then added to the reaction flasks. Reactions were stopped at the corresponding reaction times by cooling the flasks and halting agitation. As glycerol is generated during the transesterification reaction, two phases are spontaneously separated (Figure 2.5). The upper phase contained the esters formed, while the excess alcohol and the catalyst were dragged to the glycerol phase in the bottom of the flask. Distilled water was added to each flask in order to wash the ester phase and remove any rests of catalyst, alcohol and soap. All samples were centrifuged to accelerate phase separation and the aqueous-glycerol phase was removed.

2.2.4. PRODUCT CHARACTERISATION

The transesterification kinetics was followed by the changes in viscosity, and the content in esters, mono-, di-, triglycerides, and glycerol.

The kinematic viscosity was measured at 40ºC using a Cannon-Fenske viscometer for transparent liquids, previously calibrated with distilled water, according to European standard test method EN ISO 3104. All the viscosities were measured at least in triplicate. The dynamic viscosity is obtained as the product of kinematic viscosity and density. European standard test method (EN ISO 3675) was used to determine the density of all the samples at 40ºC.

The ester content was analysed by gas chromatography and based on European standard test method (EN 14103). The equipment used was an Agilent 6850 gas chromatograph, with a split/splitless inlet, a FID detector and a HP-INNOWax column.

The contents in free and total glycerol and in mono-, di- and triglycerides were also analysed by gas chromatography and based on European standard test method (EN 14105). The equipment used was an Agilent 6850 gas chromatograph, with an on-column inlet, a FID detector and a DB-5 column.

2.2.5. RESULTS AND DISCUSSION

2.2.5.1. PRODUCT APPEARANCE

Table 2.3 summarises all experiments conducted with unrefined *Cynara cardunculus* oil, and the appearance of the transesterified product. Only those experiments where phase separation was clear along the reaction (flasks with final reaction times varying from 10 to 90 minutes), were considered as presenting phase separation. Those experiments where only some of the samples (in many cases, only the 90 minutes reaction time) presented separation of phases, or where separation was not clear (soap or gel presence) after washing and centrifuging steps, were considered as presenting no phase separation.

The high FFA content is the mayor cause of experiments failure (unclear or not observed phase separation). Both NaOH and KOH are consumed by the FFA neutralisation reaction, and only small amounts of catalyst are left for the transesterification reaction. When NaOH was used, only experiments with intermediate catalyst concentration (1.0 %) were valid for all the temperatures and reaction times tested. Lower amounts of catalyst (0.5 %) were completely neutralised by the FFA present. The final product obtained in those cases contained a superior light phase (unreacted methanol), and an inferior oil+soap+gel phase. The lower phase presented a high viscosity, even after washing and centrifugation steps, indicating the presence of high amounts of unreacted oil. When higher amounts of catalyst were used (1.5 %), the formation of soaps and emulsions was important, impeding phase separation in most of the cases. For experiments were the methanol ratio was higher (7.5:1 and 9:1), phases could be separated after washing and centrifuging steps, but only for high reaction times (after 30 minutes). When KOH was used, higher amounts of catalyst (1.5 %) and reaction temperatures (60ºC) were needed to obtain separated phases. The last indicates that potassium soaps probably form more stable emulsions than sodium soaps, thus impeding the separation of the ester phase.

Both NaOH and KOH are insoluble in the longer chain alcohols tested (iso-propanol and tert-butanol), even after catalyst pulverisation and long time heating and agitation of the catalyst-alcohol mixture. Thus, sulphuric acid was used as catalyst for transesterification with those alcohols. As acid catalysed transesterification is slower than basic catalysis, longer reaction times were used in these cases.

Table 2.3. Reaction conditions for the transesterification of *Cynara cardunculus* oil

Alcohol (molar ratio)	Catalyst (% w/w)	Temperature (ºC)	Phase separation
Methanol (6:1)	NaOH (0.5%)	25 / 40 / 60	No phase separation was observed
Methanol (6:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (6:1)	NaOH (1.5%)	25 / 40 / 60	No phase separation was observed
Methanol (6:1)	KOH (0.5%)	25 / 40 / 60	No phase separation was observed
Methanol (6:1)	KOH (1.0%)	25 / 40 / 60	No phase separation was observed
Methanol (6:1)	KOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 60ºC
Methanol (7.5:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed only at 25 and 40ºC
Methanol (7.5:1)	NaOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 40ºC, and after 40 minutes reaction
Methanol (9:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed only at 25 and 40ºC
Methanol (9:1)	NaOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 40ºC, and after 40 minutes reaction
Ethanol (6:1)	NaOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (6:1)	KOH (1.0%)	25 / 60 / 75	No phase separation was observed
Iso-propanol (15:1)	NaOH (1.0%)	80	NaOH is not soluble in iso-propanol
Iso-propanol (15:1)	KOH (1.0%)	80	KOH is not soluble in iso-propanol
Iso-propanol (15:1)	H_2SO_4 (1.0%)	25 / 80	Phase separation was observed only at 80ºC
Tert-butanol (15:1)	NaOH (1.0%)	80	NaOH is not soluble in tert-butanol
Tert-butanol (15:1)	KOH (1.0%)	80	KOH is not soluble in tert-butanol
Tert-butanol (15:1)	H_2SO_4 (1.0%)	25 / 80	No phase separation was observed

2.2.5.2.   PHYSICAL PROPERTIES

Only for those experiments were two clear separated phases were obtained, the density and viscosity of the ester phase was measured. Table 2.4 presents the density and the viscosity of the samples with longer reaction times, both measured at 40ºC.

Table 2.4. Densities and viscosities of biodiesel from unrefined *Cynara cardunculus* obtained at different reaction conditions

Nº	Reaction conditions	Density (kg/m^3)	Viscosity (mPas)
1	6:1 methanol ratio, 1% NaOH, 25ºC	880	4.17
2	6:1 methanol ratio, 1% NaOH, 40ºC	880	4.35
3	6:1 methanol ratio, 1% NaOH, 60ºC	885	4.44
4	7.5:1 methanol ratio, 1% NaOH, 25ºC	882	3.88
5	7.5:1 methanol ratio, 1% NaOH, 40ºC	879	3.63
6	7.5:1 methanol ratio, 1.5% NaOH, 40ºC	880	4.02
7	9:1 methanol ratio, 1% NaOH, 25ºC	880	3.54
8	9:1 methanol ratio, 1% NaOH, 40ºC	876	3.28
9	9:1 methanol ratio, 1.5% NaOH, 40ºC	880	3.39
10	6:1 methanol ratio, 1.5% KOH, 60ºC	900	8.08

The density and the viscosity for most of the experiments satisfy European specifications (see 3. 2.1). However, the high values obtained for experiment 10 indicate that the reaction was not complete for those conditions and that high amounts of unreacted glycerides are still present.

The evolution of viscosity was followed for experiments 1 to 9 where NaOH was used as catalyst. Figure 2.6 shows that after 10 minutes reaction, the viscosity of the product obtained is much lower than the viscosity of the original oil.
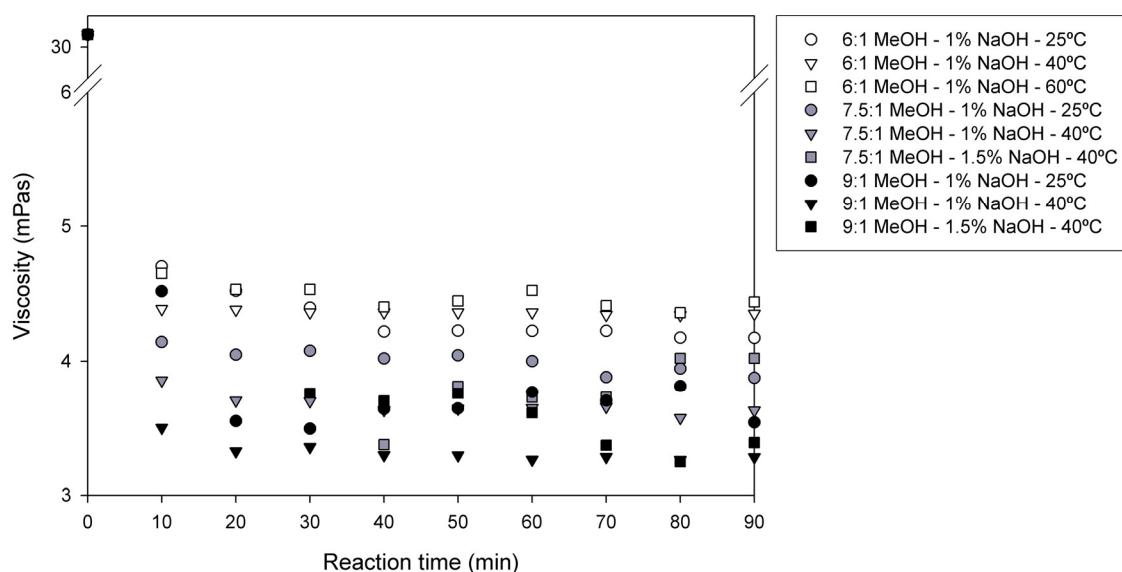
Figure 2.6. Viscosity evolution during the transesterification reaction

The viscosity decreases with higher methanol to oil molar ratio, and higher temperatures, but it increases with higher catalyst concentrations.

2.2.5.3. COMPOSITION OF THE PRODUCT OBTAINED

Figure 2.7 shows the evolution of the methyl esters content for the transesterification reaction performed for the different experiment conditions. During the first ten minutes of the reaction, the ester content reaches about 80 % for all the conditions tested. Higher reaction times do not produce an important increase in the esters yield. The low methyl esters content obtained for the final product may be due to the high FFA present in the original oil.
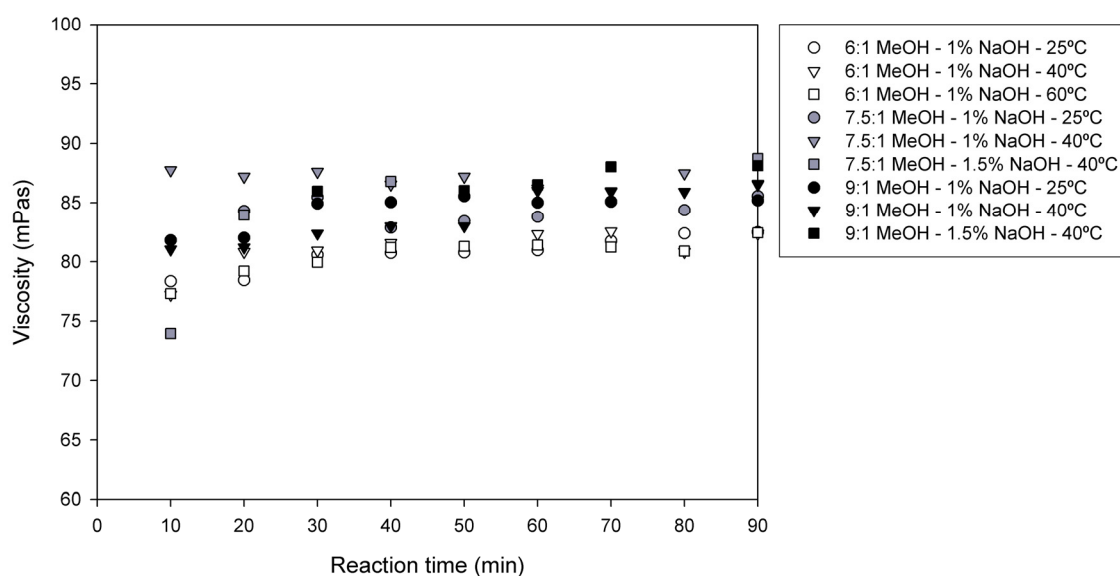
Figure 2.7. Ester yield for the transesterification of *Cynara cardunculus* oil under different reaction conditions.

2.2.6.   CONCLUSIONS

The highest methyl ester yield obtained was about only 88 %, indicating that the high FFA content affects the transesterification yield in different ways. The FFA neutralisation consumes a high percentage of the catalyst used and leaves only small amounts of catalyst available for the transesterification reaction. In addition, the formation of soaps difficults the separation of the esters formed from the glycerol phase. This shows the need of a pre-treatment step in order to decrease the initial acid value of the oil.

2.3. ACID CATALYSED PREESTERIFICATION

2.3.1. INTRODUCTION

The main drawback of alkaline catalysts is their sensitivity to the FFA contained in the oil *(Mittelbach 2004)*. FFA react with basic catalysts forming soaps, that lower the catalytic activity, reduce the reaction yield and emulsify the final product impeding glycerol separation *(Canakci 2001)*. Thus, the acid number of fats and oils has to be reduced to values below 1 mg KOH/g (almost 2 % FFA) prior to alkali catalysed transesterification. Deacidification methods include simple neutralisation with alkali, distillation, extraction with solvents and acid catalysed pre-esterification *(Mittelbach 2004)*. When the acid value is low, alkaline catalysts are used in small increased amounts in order to neutralise the FFA, that are then separated as wastes. However when the FFA content increases this becomes undesirable because of the loss of feedstock and the formation of emulsions *(Canakci 2001)*. The alkali-catalysed transesterification of high FFA oils, requires a supplement of catalyst to neutralise the FFA. A second two-step transesterification is needed in this cases in order to obtain a higher conversion. The higher the acidity of the oil, the smaller the reaction yield. The two-step alkaline-catalysed reaction is a good method for biodiesel production when the FFA content is smaller than 3 %. Higher values show no ester formation *(Dorado 2002)*. The acid catalysed pre-esterification is the most widely used method, when using waste oils, animal fats, or oils with a high FFA content. The oil is treated with methanol or ethanol in the presence of an acid catalyst, usually sulphuric acid, in order to esterify the FFA. The resulting oil has to be treated to remove the water formed during the reaction and the acid catalyst. Then, traditional alkali catalysed transesterification can be applied *(Mittelbach 2004)*. Thus, the biodiesel production process needs the use of two or three steps. Firstly, the pre-esterification of the FFA with an acid catalyst, and secondly, the one or two step transesterification of the triglycerides with an alkaline catalyst. The pre-esterification rate depends on the amount of catalyst, the reaction duration and the content of FFA in the oil *(Sendzikiene 2004)* .

Acid catalysts alone have the advantage of esterifying the FFA contained in the oil and enabling the production of long- or branched- chain esters. However they require higher amounts of alcohol, pressures, temperatures and reaction times. Acid catalysts are too slow to be practical for converting tryglicerides to biodiesel. However they appear to be quite effective at converting FFA to esters *(Canakci 2001)*. That is why acid catalysed

transesterification are seldom used as the sole reactions in industrial biodiesel production. They are frequently used as preliminary steps for the deacidification of fatty materials followed by traditional alkaline operation. Because of its low price, sulphuric acid is the most commonly acid catalyst used. Although it is corrosive and may cause dark colouring in the ester product, its hygroscopicity is important to remove released water from the reaction mixture *(Mittelbach 2004)*.

There is a wide variety of high FFA oils that are available in large quantities. These unrefined oils, unsuitable for human consumption, are usually cheaper than refined ones, making them good candidates for biodiesel production. In India, a two step process has been developed for the treatment of rubber seed oil with an acid value of 34 mg KOH/g. In a first step, they reduce the acid value to 4 mg KOH/g, using 0.5 % (v/v) of sulphuric acid and 6:1 methanol to oil molar ratio, after 30 minutes at 50ºC. Second step is the alkaline transesterification of the first step product, after removing impurities, obtaining a biodiesel that reasonably agrees with ASTM standards. The best results where obtained for a 9:1 methanol to oil molar ratio and 0.5 % (w/w) NaOH after 30 minutes at 50ºC *(Ramadhas 2005)*.

However other authors suggest that the acid value must not be higher than 1 or 2 mg KOH/g *(Ghadge 2005, Ma 1998)* in order to favour phase separation of glycerol. In some cases a two-step acid esterification is needed prior to alkaline reaction. Experiments with crude mahua oil *(Ghadge 2005)* show that the acid value can be reduced from 38 to 5 mg KOH/g in a first acid esterification step, using 1 % (v/v) sulphuric acid and 0.30 methanol to oil volume fraction, after 1 hour at 60ºC. The water produced during the reaction prevents the reduction of the acid value after 1 hour. Because of that, a second step is needed to reduce acid value from 5 to less than 2 mg KOH/g after removing water, acid and excess methanol from the first one, and using 1 % (v/v) sulphuric acid and 0.35 methanol to oil volume fraction, after 1 hour at 60ºC. Thus, alkaline transesterification can be carried out as a third step, using 0.7 % (w/w) KOH and 0.25 methanol to oil volume fraction, after 30 minutes at 60ºC.

The purpose of the present chapter is to develop a method for *Cynara cardunculus* oil pre-treatment, in order to reduce the initial acid value of 11.63 mg KOH/g to less than 1.0 mg KOH/g

2.3.2. PRE-TREATMENT METHODOLOGY AND REACTION CONDITIONS

To reduce the acid value of the oil, acid catalysed reactions with methanol were performed at different temperatures, methanol to oil molar ratios, catalyst (H_2SO_4) concentrations and reaction time. Table 2.5 details the different operation conditions.

Table 2.5. Reaction conditions for *Cynara cardunculus* oil preesterification

Methanol to oil molar ratio	4.5:1 / 6:1 / 7.5:1 / 9:1
H_2SO_4 (% v/v)	0.5 / 1.0 / 1.5
Temperature (ºC)	40 / 60
Reaction time (minutes)	45 / 60 / 75 / 90

All experiments were conducted in 250 ml glass flasks, kept under permanent agitation in a water bath at the desired temperature. 50 ml of *Cynara cardunculus* oil were preheated in each flask until they reached the experimental temperature. Methanol and concentrated H_2SO_4 were added to the flasks in the correspondent proportions. Reactions were stopped at different times by cooling the flasks and halting agitation. As some water is generated during the esterification reaction, two phases are spontaneously separated. The oily phase contains the esters formed, while the excess methanol and the H_2SO_4 are dragged to the aqueous phase in the bottom of the flask. All samples are centrifuged to accelerate phase separation and the aqueous phase is removed. However, not all the excess methanol is dragged by the water, and, as methanol density is lower than that of oil, some alcohol is allowed to keep on the oil's surface, or in small bubbles along the oily phase, usually in samples with higher methanol to oil molar ratios. To completely separate methanol and assure the removal of catalyst, the samples were washed with distilled water and centrifuged. This process was repeated until the pH of the removed water phase was neutral, and no methanol bubbles were observed. The acid value of the oil phase was then determined by European standard test method (EN 14104).

2.3.3. PRODUCT CHARACTERISATION

During the pre-treatment process, some triglycerides are transesterified and form methyl esters and glycerol. The transesterification kinetics was followed by the changes in the acid value, and the content in esters, mono-, di-, triglycerides, and glycerol.

The acid value of the samples was determined by European standard test method (EN 14104).

The ester content was analysed by gas chromatography and based on a standard test method (UNE-EN 14103). The equipment used was an Agilent 6850 gas chromatograph, with a split/splitless inlet, a FID detector and a HP-INNOWax column.

The contents in free and total glycerol and in mono-, di- and triglycerides were also analysed by gas chromatography and based on a standard test method (UNE-EN 14105). The equipment used was an Agilent 6850 gas chromatograph, with an on-column inlet, a FID detector and a DB-5 column.

2.3.4. RESULTS AND DISCUSSION

The evolution of the acid value during the preesterification reactions is shown in figures 2.8 and 2.9.

At 40ºC, the acid value decreases for higher methanol to oil molar ratios, and higher sulphuric acid concentrations. However, after 60 minutes reaction, changes in the acid value with time have low significance. To reach the limit of 1 mg KOH/g oil, the higher methanol to oil molar ratios are needed. If we want to reduce the methanol consumption to the minimum, higher sulphuric acid concentrations are needed.

At 60ºC the acid value decreases for higher methanol to oil molar ratios. However, sulphuric acid concentrations and reaction times have almost no effect in the acid values. At this temperature, only experiments with lower methanol to oil molar ratios (4.5:1) exceed acid value limit established. It was demonstrated that 6:1 methanol to oil molar ratio, 0.5 % H_2SO_4, and 60 minutes reaction are enough to reduce the acid value to the limits required.
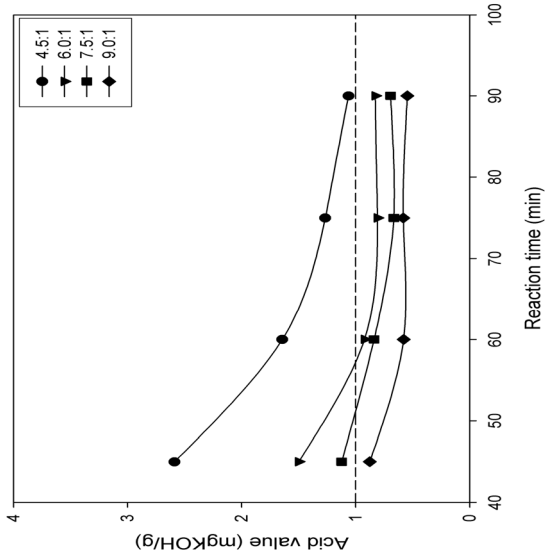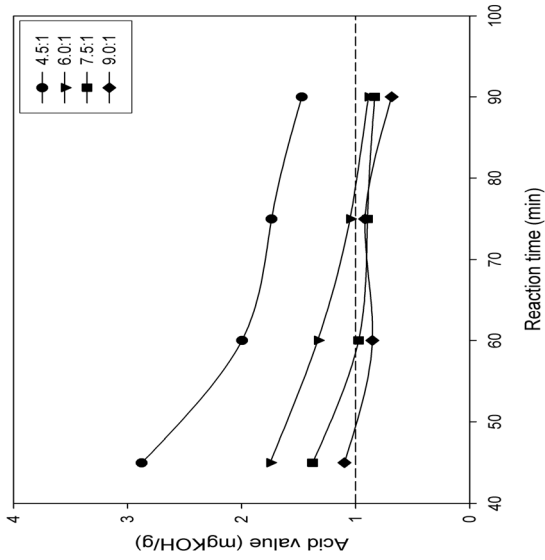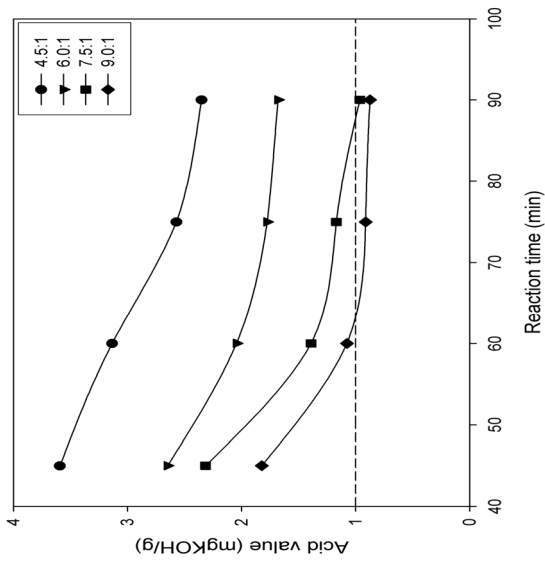
Figure 2.8. Acid value evolution for the preesterification of *Cynara cardunculus* oil at 40ºC, using 0.5, 1.0 and 1.5 % H_2SO_4 for figures a, b and c respectively.
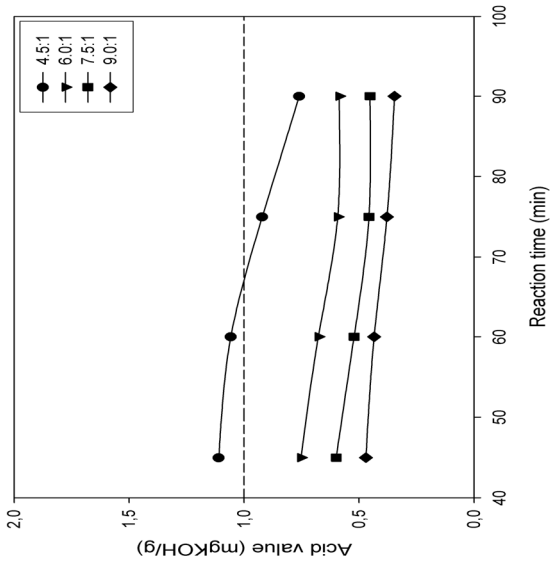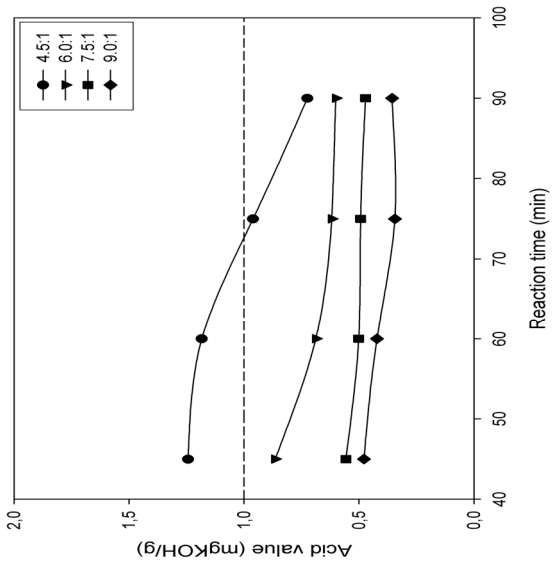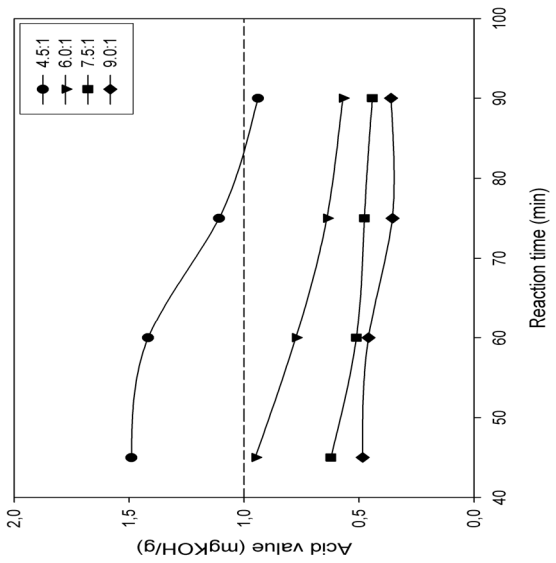
Figure 2.9. Acid value evolution for the preesterification of *Cynara cardunculus* oil at 60°C, using 0.5, 1.0 and 1.5 % H_2SO_4 for figures a, b and c respectively.

The best reaction conditions were obtained for 60ºC, using a methanol to oil molar ratio of 6:1 and 0.5 % sulphuric acid as catalyst. The evolution of the ester content, together with the mono-, di-, triglycerides and FFA is shown in figure 2.10. The maximum ester yield obtained was of about 6.6 %. These results indicate that the presence of water, formed during the esterification reaction, inhibits the methyl esters production, both from the FFA present and from the triglycerides. However, the ester percentage obtained and the low acid values reached after the reaction, show that almost all the FFA present in the original oil have been successfully esterified.
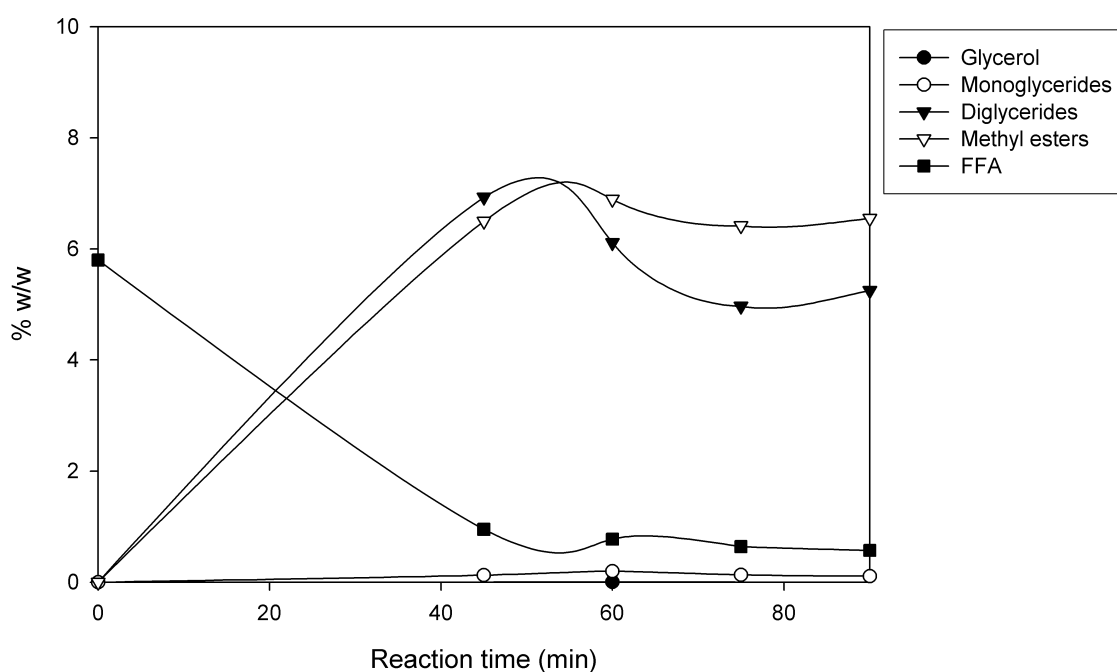
Figure 2.10. Evolution of the glycerol content, mono and diglycerides, FFA and methyl esters for the preesterification of *Cynara cardunculus* oil at 60ºC using 6:1 methanol to oil molar ratio and 0.5 % H_2SO_4.

2.3.5. REACTION KINETICS

2.3.5.1. THE REACTION MECHANISM

The overall transesterification reaction, together with the esterification of the free fatty acids, are described in equations 2.1 and 2.2. The transesterification occurs by the combination of 3 molecules of methanol per molecule of triglycerides present in the original oil, while the esterification of the free fatty acids occurs by the combination of a molecule of free fatty acid with a molecule of methanol to form water and methyl esters.

$$TG + 3\,MeOH \rightleftharpoons GL + 3\,FAME \tag{2.1}$$

$$FFA + MeOH \rightleftharpoons H_2O + FAME \tag{2.2}$$

Although the initial reactants (oil and methanol) are inmiscible and two phases are present at the beginning of the reaction, as soon as the mono- and diglycerides are produced, they act as co-solvents. Thus the reaction system can be considered as a pseudo-homogeneous one. Equation system 2.3 describes the transesterification and esterification mechanism, consisting of a series of four reversible reactions.
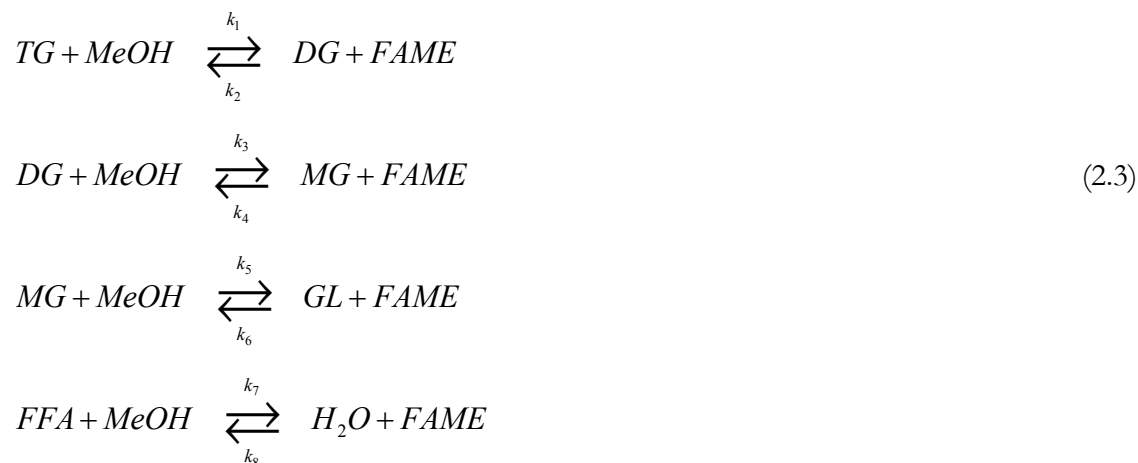
$$TG + MeOH \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DG + FAME$$

$$DG + MeOH \underset{k_4}{\overset{k_3}{\rightleftharpoons}} MG + FAME \tag{2.3}$$

$$MG + MeOH \underset{k_6}{\overset{k_5}{\rightleftharpoons}} GL + FAME$$

$$FFA + MeOH \underset{k_8}{\overset{k_7}{\rightleftharpoons}} H_2O + FAME$$

where:

TG: triglycerides

DG: diglycerides

MG: monoglycerides

MeOH: methanol

FAME: fatty acid methyl esters

GL: glycerol

FFA: free fatty acids

H_2O: water

k_1, k_3, k_5 and k_7 : specific forward reaction rate constants

k_2, k_4, k_6 and k_8 : specific reverse reaction rate constants

The forward and reverse reaction rate constants are related by the reaction equilibrium constants:

$$K_{12} = \frac{k_1}{k_2} \quad , \quad K_{34} = \frac{k_3}{k_4} \quad , \quad K_{56} = \frac{k_5}{k_6} \quad , \quad K_{78} = \frac{k_7}{k_8} \tag{2.4}$$

If the four reversible reactions are assumed to be elementary reactions and they all follow a second order reaction kinetics, the following kinetic equations are obtained:

$$\frac{d[TG]}{dt} = -k_1[TG][MeOH] + k_2[DG][FAME]$$

$$\frac{d[DG]}{dt} = k_1[TG][MeOH] - k_2[DG][FAME] - k_3[DG][MeOH] + k_4[MG][FAME]$$

$$\frac{d[MG]}{dt} = k_3[DG][MeOH] - k_4[MG][FAME] - k_5[MG][MeOH] + k_6[GL][FAME]$$

$$\frac{d[FFA]}{dt} = -k_7[FFA][MeOH] + k_8[H_2O][FAME] \tag{2.5}$$

$$\frac{d[FAME]}{dt} = k_1[TG][MeOH] - k_2[DG][FAME] + k_3[DG][MeOH] - k_4[MG][FAME]$$
$$+ k_5[MG][MeOH] - k_6[GL][FAME] + k_7[FFA][MeOH] - k_8[H_2O][FAME]$$

$$\frac{d[MeOH]}{dt} = -k_1[TG][MeOH] + k_2[DG][FAME] - k_3[DG][MeOH] + k_4[MG][FAME]$$
$$- k_5[MG][MeOH] + k_6[GL][FAME] - k_7[FFA][MeOH] + k_8[H_2O][FAME]$$

$$\frac{d[GL]}{dt} = k_5[MG][MeOH] - k_6[GL][FAME]$$

$$\frac{d[H_2O]}{dt} = k_7[FFA][MeOH] - k_8[H_2O][FAME]$$

This differential equation system must be solved in order to obtain the different specific reaction rate constants and verify the accuracy of the proposed model. Due to the complexity of the problem this must be solved by the use of numerical methods.

The concentration evolution of the mono-, di-, triglycerides and methyl esters was obtained from the chromatographic analysis and recalculated by the volume of methanol added. The concentration of the FFA was obtained by the acid value method.

The initial concentrations of the mono-, di-, triglycerides and FFA were re-calculated after the addition of the corresponding methanol amount while the initial concentrations of glycerol, water and methyl esters were considered to be zero.

The concentrations of methanol, water and glycerol along the reaction were calculated through a material balance for the reaction where:

$$[GL] = ([TG]_0 - [TG]) + ([DG]_0 - [DG]) + ([MG]_0 - [MG]) \tag{2.6}$$

$$[MeOH] = [MeOH]_0 - ([FAME] - [FAME]_0) \tag{2.7}$$

$$[H_2O] = [FFA]_0 - [FFA] \tag{2.8}$$

2.3.5.2.   NUMERICAL MODELLING

Two different computing programs (GEPASI 3.30 and POLYMATH 6.0) were used to solve the problem.

The program GEPASI is a software package intended for modelling chemical and biochemical reaction systems. The user must supply the program with information about the stoichiometric structure of the reaction mechanism, kinetics of each reaction, initial concentration of all chemical species and any additional function. The program then builds the differential equations that govern the behaviour of the system and solves them. The program is able to simulate the steady-state and time-course behaviour of reactions and fit the models to data, optimising any function. Thus, the simulated theoretical model can be compared with the experimental data for the concentration evolution of the different reactants.

To solve the present problem, the program GEPASI was provided with the mechanism described in equation 2.3, initial concentrations of all reactants, concentration evolution with time of all reactants and initial estimations for the ranges of the forward reaction kinetic constants. The equilibrium relations between the forward and reverse rate constants (equation 2.4) were also provided.

The program output included the optimal values for the specific rate constants (those that derived in the minimum value of sums of squares of deviations) and the theoretical concentration evolution for the different reactants calculated by the integration of the model (equation system 2.5).

The program POLYMATH 6.0 was then use to verify the concordance between the experimental data and the proposed model, using the specific reaction rate constants obtained from GEPASI. POLYMATH is a system of programs useful for solving linear, non-linear and differential systems of equations. It can also obtain data regressions. The differential equations solver program employs Runge-Kutta method to solve the differential equation systems.

2.3.5.3.   SPECIFIC RATE CONSTANTS

The reaction kinetics was evaluated for all the different reaction conditions. As the acid value was used to choose the optimal reaction conditions, the reaction conducted at 60ºC with 6:1 methanol to oil molar ratio and using 0.5 % H_2SO_4 as catalyst, was analysed in detail along this chapter. The kinetic rate constants were determined for this reaction and compared with the reaction conducted at 40ºC. The results for the rest of the experiments are detailed in appendix B.

The initial data provided to program GEPASI were the initial concentration for all the reactants, the equilibrium rate constants and the estimated forward rate constants, detailed in table 2.6.

The provided estimated values for the forward rate constants were obtained from the numerical integration of equations 2.9 to 2.12.

$$k_1 = \frac{d[TG]/dt}{\left(\dfrac{[DG][FAME]}{K_{12}} - [TG][MeOH]\right)} \qquad (2.9)$$

$$k_3 = \frac{d[DG]/dt - \left(k_1[TG][MeOH] - k_2[DG][FAME]\right)}{\left(\dfrac{[MG][FAME]}{K_{34}} - [DG][MeOH]\right)} \qquad (2.10)$$

$$k_5 = \frac{d[MG]/dt - (k_3[DG][MeOH] - k_4[MG][FAME])}{\left(\dfrac{[GL][FAME]}{K_{56}} - [MG][MeOH]\right)} \qquad (2.11)$$

$$k_7 = \frac{d[FFA]/dt}{\left(\dfrac{[H_2O][FAME]}{K_{78}} - [FFA][MeOH]\right)} \qquad (2.12)$$

Experimental data for the concentration evolution of all the reactants was provided to the program together with the suggested ranges for k_1, k_3, k_5 and k_7.

Table 2.6. Initial conditions for the reaction conducted at 40 and 60ºC using 6:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.

Parameter	Units	40ºC	60ºC
$[TG]_0$	mol/l	0.741	0.741
$[DG]_0$	mol/l	0.0331	0.0331
$[MG]_0$	mol/l	0.0018	0.0018
$[FAME]_0$	mol/l	0.0	0.0
$[MeOH]_0$	mol/l	4.45	4.45
$[GL]_0$	mol/l	0.0	0.0
$[FFA]_0$	mol/l	0.149	0.149
$[H_2O]_0$	mol/l	0.0	0.0
K_{12}	-	0.0018	0.0032
K_{34}	-	0.0012	0.0014
K_{56}	-	0.19	0.01
K_{78}	-	1.46	2.83
k_1	l/mol.min	0.016	0.015
k_3	l/mol.min	0.37	0.06
k_5	l/mol.min	6.95	1.2
k_7	l/mol.min	0.0032	0.026

The results obtained from the program were the final concentrations of all the reactants, and the calculated forward and reverse kinetic rate constants. All these parameters are detailed in table 2.7.

Table 2.7. Results provided by GEPASI for the reaction conducted at 40 and 60ºC using 6:1 methanol molar ration and 0.5 % H_2SO_4 as catalyst.

Parameter	Units	40ºC	60ºC
$[TG]_{final}$	mol/l	0.73	0.71
$[DG]_{final}$	mol/l	0.04	0.06
$[MG]_{final}$	mol/l	0.0015	0.002
$[FAME]_{final}$	mol/l	0.144	0.17
$[MeOH]_{final}$	mol/l	4.3	4.28
$[GL]_{final}$	mol/l	0.008	0.0005
$[FFA]_{final}$	mol/l	0.036	0.0082
$[H_2O]_{final}$	mol/l	0.113	0.14
SSD	-	1.6 e-5	1.2 e-5
k_1	l/mol.min	0.016	0.0011
k_3	l/mol.min	0.37	0.058
k_5	l/mol.min	6.95	1.2
k_7	l/mol.min	0.0038	0.008
k_2	l/mol.min	8.9	0.34
k_4	l/mol.min	301	41
k_6	l/mol.min	36	118
k_8	l/mol.min	0.0026	0.0028

Where SSD is the sum of square deviations

Figures 2.11 and 2.12 show the comparison of the theoretical concentration evolution calculated for the model and the experimental data.
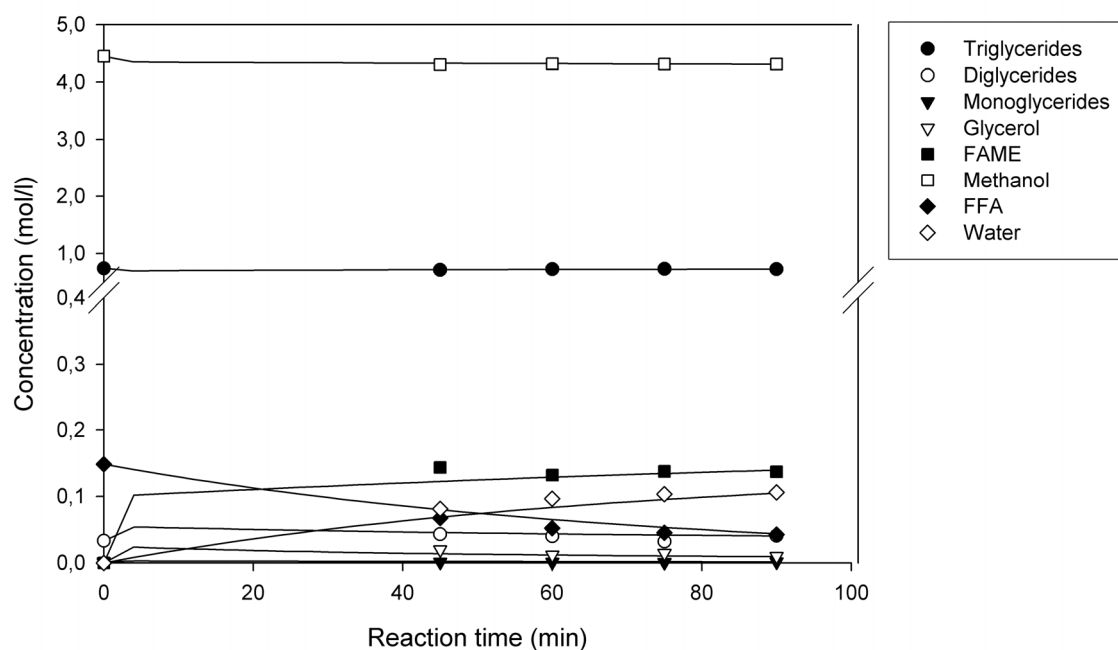
Figure 2.11. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40°C using 6:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.
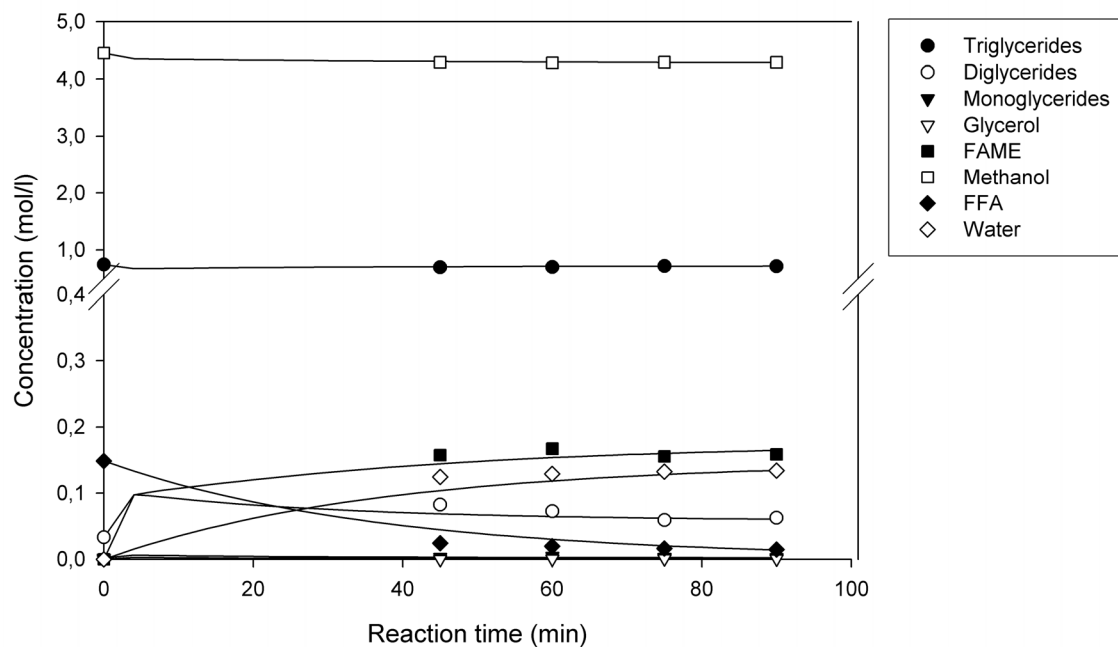
Figure 2.12. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60°C using 6:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.

50

2.3.5.4. EFFECT OF TEMPERATURE

The influence of temperature on the specific reaction rate constants is determined form the Arrhenius equation and its integrated form (equations 2.13 and 2.14).

$$\frac{d\ln k}{dT} = \frac{E_a}{RT^2} \tag{2.13}$$

$$k = A \cdot e^{\left(-\frac{E_a}{RT}\right)} \tag{2.14}$$

Where A is the pre-exponential factor, E_a is the activation energy of the reaction and R is the gas constant. The Arrhenius parameters, A and E_a, can be determined from the logarithmic form of Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \tag{2.15}$$

By plotting the logarithm of the rate constant as a function of T^{-1}, the pre-exponential factor, A, can be obtained from the intercept and the activation energy, E_a, can be obtained from the slope.

For the preesterification reaction conducted using a 6:1 methanol to oil molar ratio and 0.5% H_2SO_4, the effect of temperature on the specific rate constants is shown in figure 2.13. Table 2.8 presents the results for A and E_a, for the different rate constants.

Though most of the specific rate constants decrease with temperature, the specific rate constants that affect the reaction from FFA to FAME and from FAME to MG. This results indicate that the preesterification reaction is favoured by an increase in temperature, though the final ester conversion may decrease. This explains the fact that the reaction conditions that conducted to the lowest FFA content don not present the highest FAME yield. However, as the main objective of the preesterification step was to decrease the FFA content, the optimal preesterification conditions are those that give the lowest acid value, with the lowest methanol to oil molar ratio and catalyst concentration.

The effect of temperature for all the reaction conditions tested are detailed in appendix B.
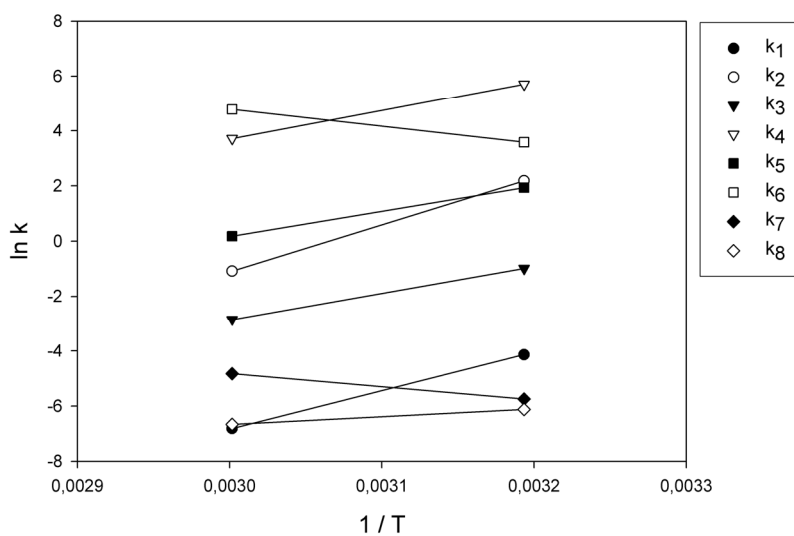
Figure 2.13. Effect of temperature on the reaction rate constants for the reaction conducted using 6:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst

Table 2.8. Arrhenius parameters for the reaction conducted using 6:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst

Specific rate constant	A (l/mol.min)	E_a (J/mol)
k_1	6.85×10^{-22}	-1680
k_2	1.95×10^{-23}	-2052
k_3	1.30×10^{-14}	-1167
k_4	1.08×10^{-12}	-1253
k_5	1.37×10^{-12}	-1102
k_6	$1.52 \times 10^{+10}$	748
k_7	$1.36 \times 10^{+4}$	575
k_8	2.53×10^{-7}	-341

2.3.6. CONCLUSIONS

The most important parameter to be optimised was the acid value. Thus, the best reaction conditions for the pre-treatment step are chosen from the acid value evolution. Although for the reactions conducted at 40ºC, in some cases the acid value was reduced below 1 mgKOH/g, this only occurred at the highest methanol amounts, catalyst concentrations and reaction times.

On the other hand, for almost all the reactions conducted at 60ºC the acid value was below the recommended limits. It was demonstrated that at 60ºC, a 6:1 methanol to oil molar ratio, 0.5 % H_2SO_4 and 60 minutes reaction time are the best preesterification conditions, taking into account the achievement of the objective (an acid value < 1 mgKOH/g) and the economy of the process.

2.4. TRANSESTERIFICATION OF THE PRE-TREATED OIL

2.4.1. REACTION METHODOLOGY AND CONDITIONS

Unrefined *Cynara cardunculus* oil was pre-treated in two ways for further alkaline transesterification: degumming and pre-esterification.

2.4.1.1. OIL DEGUMMING

The first pre-treatment step was the oil degumming, in order to remove any phosphatides present in the original oil. This step was conducted at 60ºC, adding 15 ml distilled water per litre of oil, and keeping the oil under agitation during 60 minutes. The oil was centrifuged at 4600 rpm during 15 minutes in order to separate the water with the dissolved phosphatides. As not all the water is removed during centrifugation and some small amounts may remain in the oily phase, the oil was then heated at 105ºC during 1 hour in order to evaporate any rests of water. The water content of the oil was measured using a Karl Fisher titrator, before and after the heating steps in order to ensure a final low water amount.

2.4.1.2. OIL PRE-ESTERIFICATION

The second pre-treatment step was the oil preesterification in order to reduce the FFA content. This step was conducted at 60ºC, using a 6:1 methanol to oil molar ratio, 0.5 % H_2SO_4 , and keeping the oil under agitation during 60 minutes. These pre-treated oil was washed with distilled water in order to remove the H_2SO_4 and the excess methanol. Washing steps where repeated until the separated water presented a neutral pH. The oil was then centrifuged at 4600 rpm during 15 minutes in order to separate the rests of water. As not all the water is removed during centrifugation and some small amounts may remain in the oily phase, the oil was then heated at 105ºC during 1 hour in order to evaporate any rests of water. The water content of the oil was measured using a Karl Fisher titrator, before and after the heating steps in order to ensure a final low water amount.

2.4.1.3.   ALKALINE TRANSESTERIFICATION

Different short chain alcohols were used, such as methanol and ethanol. Reactions with methanol were performed at room temperature (25ºC), 40 and 60 ºC. Reactions with ethanol were performed at room temperature (25ºC), 40 and 75 ºC. Alcohol to oil molar ratios ranging from 6:1 to 9:1 were used. The catalysts used were NaOH and KOH, with concentrations of 0.5, 1.0 and 1.5 % by weight of oil. The maximum reaction time was of 90 minutes.

All experiments were conducted in 250 ml glass flasks, kept under permanent agitation in a water bath at the desired temperature. Each flask contained 50 ml of pre-treated *Cynara cardunculus* oil, that was heated until it reached the experimental temperature. The alcohol and catalyst were premixed at the correspondent proportions, and then added to the reaction flasks. Reactions were stopped after 90 minutes by cooling the flasks and halting agitation. Distilled water was added to each flask in order to wash the ester phase and remove any rests of catalyst, alcohol and soap. All samples were centrifuged to accelerate phase separation and the aqueous-glycerol phase was removed.

Reaction kinetics was followed for some of the reactions by using a 1000 ml glass reactor, kept under permanent agitation in a water bath at the desired temperature (figure 2.14). For each experiment, 500 ml of pre-treated *Cynara cardunculus* oil were introduced into the reactor, that was preheated until it reached the experimental temperature. The alcohol and catalyst were premixed at the correspondent proportions, and then added to the reactor. Five ml samples were taken with a syringe from the reactor at 1, 3, 5, 7, 10, 20, 30, 40, 50, 70 and 90 minutes of reaction. Samples were put into 10 ml centrifuge tubes, in a cold bath. As glycerol is generated during the transesterification reaction, two phases are spontaneously separated. The upper phase contained the esters formed, while the excess alcohol and the catalyst were dragged to the glycerol phase in the bottom of the tube. Distilled water was added to each tube in order to wash the ester phase and remove any rests of catalyst, alcohol and soap. All samples were centrifuged to accelerate phase separation and the aqueous-glycerol phase was removed.

Figure 2.14. Reactor used for the transesterification of the pre-treated oil

2.4.2. PRODUCT CHARACTERISATION

The transesterification kinetics was followed by the changes in the esters, mono-, di-, triglycerides, and glycerol contents.

The ester content was analysed by gas chromatography and based on European standard test method (EN 14103). The equipment used was an Agilent 6850 gas chromatograph, with a split/splitless inlet, a FID detector and a HP-INNOWax column.

The contents in free and total glycerol and in mono-, di- and triglycerides were also analysed by gas chromatography and based on European standard test method (EN 14105). The equipment used was an Agilent 6850 gas chromatograph, with an on-column inlet, a FID detector and a DB-5 column.

2.4.3. RESULTS AND DISCUSSION

2.4.3.1. PRODUCT APPEARANCE

Table 2.9 summarises all experiments conducted with pre-treated *Cynara cardunculus* oil, and the appearance of the transesterified product. Only those experiments where phase separation was clear, were considered as presenting phase separation. Those experiments where no phase separation occurred or phase separation was not clear (soap or gel presence) after washing and centrifuging steps, were considered as presenting no phase separation.

Table 2.9. Reaction conditions for the transesterification of pre-treated *Cynara cardunculus* oil.

Alcohol (molar ratio)	Catalyst (% w/w)	Temperature (ºC)	Phase separation
Methanol (6:1)	NaOH (0.5%)	25 / 40 / 60	Phase separation was observed only at 40 and 60ºC
Methanol (6:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (6:1)	NaOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 40 and 60ºC
Methanol (6:1)	KOH (0.5%)	25 / 40 / 60	Phase separation was observed only at 40 and 60ºC
Methanol (6:1)	KOH (1.0%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (6:1)	KOH (1.5%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (7.5:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (7.5:1)	NaOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 40 and 60ºC
Methanol (9:1)	NaOH (1.0%)	25 / 40 / 60	Phase separation was observed for all the temperatures tested
Methanol (9:1)	NaOH (1.5%)	25 / 40 / 60	Phase separation was observed only at 60ºC
Ethanol (6:1)	NaOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (6:1)	KOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (7.5:1)	NaOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (7.5:1)	KOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (9:1)	NaOH (1.0%)	25 / 60 / 75	No phase separation was observed
Ethanol (9:1)	KOH (1.0%)	25 / 60 / 75	No phase separation was observed

Comparing this table with table 2.3, it can be observed that most of the reaction conditions that presented no phase separation for unrefined oil, present separated phases when the oil is pre-treated. Thus, both degumming and pre-esterification steps represent an improvement of reaction behaviour and final product appearance.

Although there was not phase separation for the reactions conducted with ethanol, the formation of soap or gel was also not observed. In contrast, all samples presented a clear and unique phase.

2.4.3.2.   PRODUCT COMPOSITION

The final methyl ester contents for the different reaction conditions, where phase separation was observed, are detailed in table 2.10.

Table 2.10. Final methyl ester contents for the transesterification of pre-treated *Cynara cardunculus* oil.

Methanol molar ratio	Catalyst type	Catalyst %	Temperature (ºC)	Methyl ester %
6:1	NaOH	0.5	40	10.5
6:1	NaOH	0.5	60	79.0
6:1	NaOH	1.0	25	78.3
6:1	NaOH	1.0	40	82.4
6:1	**NaOH**	**1.0**	**60**	**91.8**
6:1	NaOH	1.5	40	90.6
6:1	NaOH	1.5	60	90.7
6:1	KOH	0.5	40	8.8
6:1	KOH	0.5	60	16.5
6:1	KOH	1.0	40	12.0
6:1	KOH	1.0	60	87.7
6:1	KOH	1.5	25	82.4
6:1	**KOH**	**1.5**	**60**	**90.0**
7.5:1	NaOH	1.0	40	92.3
7.5:1	NaOH	1.0	60	92.0
7.5:1	NaOH	1.5	40	90.3
7.5:1	NaOH	1.5	60	90.5
9:1	NaOH	1.0	40	90.2
9:1	NaOH	1.0	60	91.0
9:1	NaOH	1.5	60	91.7

When NaOH was used as catalyst, the optimum methyl ester yield (91.8 %) was obtained for the reaction conducted at 60ºC, using a methanol to oil molar ratio of 6:1 and 1 % NaOH as catalyst. It is observed that when higher amounts of catalyst are used there is no increase in the methyl esters yield. When the methanol to oil molar ratio is increased, there are almost no changes in the methyl esters yield and the small increment observed for the 7.5:1 molar ratio is not significant, specially if considering the important methanol cost increment.

Figure 2.15 shows the comparison of the ester yield of this reaction with the reaction conducted under the same conditions for unrefined oil. It was obtained a difference of 9.3 % in the methyl esters yield when pre-treated oil was used, compared to the same reaction conducted with the unrefined oil.
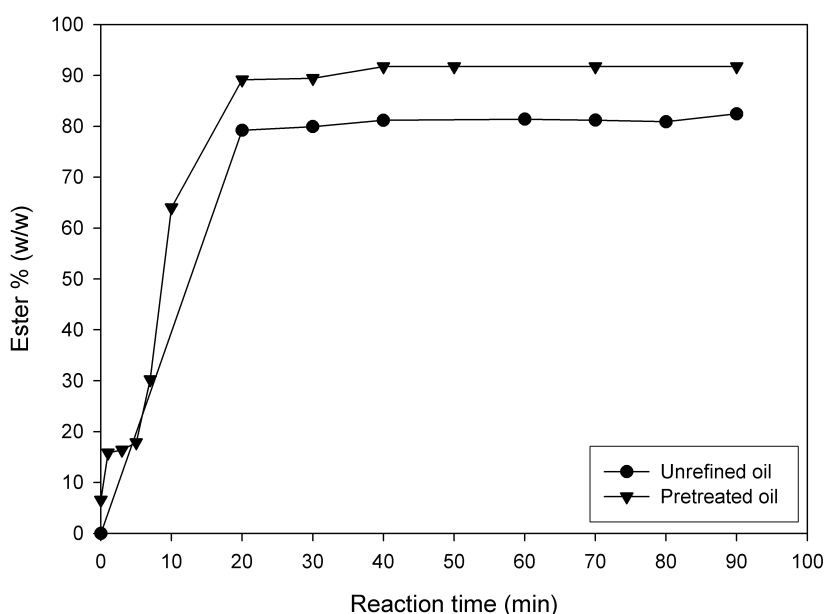
Figure 2.15. Comparison of the ester yield for the transesterification of unrefined and pre-treated oil under the optimum reaction conditions with NaOH as catalyst

When KOH was used as catalyst, the optimum methyl esters yield (89.7 %) was obtained for the reaction conducted at 60ºC, using a methanol to oil molar ratio of 6:1 and 1.5 % KOH as catalyst.

Figure 2.16 shows the comparison of the ester yield of this reaction with the reaction conducted under the same conditions for unrefined oil. It was obtained a difference of 14.0 % in the methyl esters yield when pre-treated oil was used, compared to the same reaction conducted with the unrefined oil.
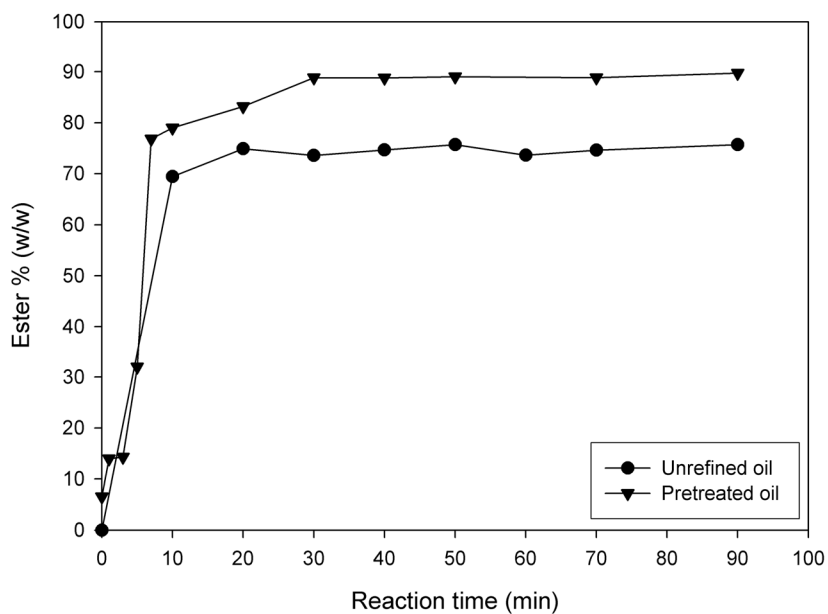
Figure 2.16. Comparison of the ester yield for the transesterification of unrefined and pre-treated oil under the optimum reaction conditions with KOH as catalyst

2.4.3.3.   REACTION KINETICS

2.4.3.3.1.   THE REACTION MECHANISM

The overall transesterification reaction, described in equation 2.16, occurs by the combination of 3 moles of alcohol per mole of triglycerides present in the original oil.

$$TG + 3\ MeOH \rightleftarrows GL + 3\ FAME \tag{2.16}$$

Although the initial reactants (oil and methanol) are inmiscible and two phases are present at the beginning of the reaction, as soon as the mono- and diglycerides are produced, they act as co-solvents. Thus the reaction system can be considered as a pseudo-homogeneous one. Equation 2.17 describes the transesterification mechanism, consisting of a series of three reversible reactions.
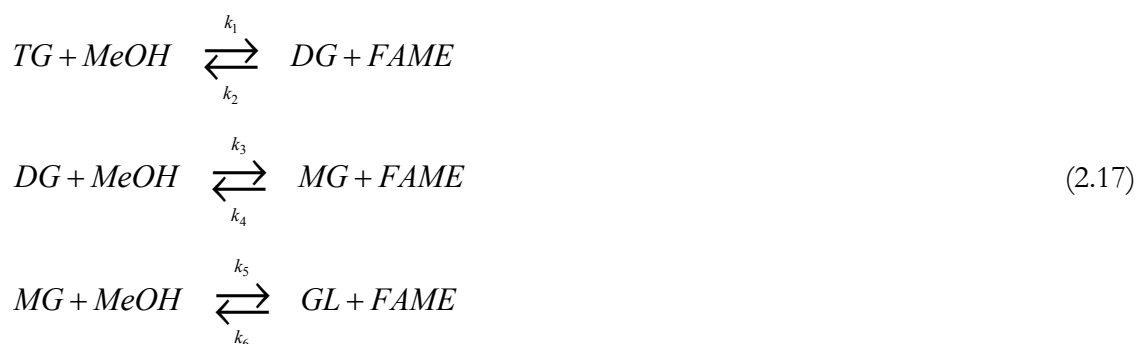
$$TG + MeOH \underset{k_2}{\overset{k_1}{\rightleftarrows}} DG + FAME$$

$$DG + MeOH \underset{k_4}{\overset{k_3}{\rightleftarrows}} MG + FAME \tag{2.17}$$

$$MG + MeOH \underset{k_6}{\overset{k_5}{\rightleftarrows}} GL + FAME$$

where:

TG: triglycerides

DG: diglycerides

MG: monoglycerides

MeOH: methanol

FAME: fatty acid methyl esters

GL: glycerol

k_1, k_3 and k_5 : specific forward reaction rate constants

k_2, k_4 and k_6 : specific reverse reaction rate constants

The forward and reverse reaction rate constants are related by the reaction equilibrium constants:

$$K_{12} = \frac{k_1}{k_2} \quad , \quad K_{34} = \frac{k_3}{k_4} \quad , \quad K_{56} = \frac{k_5}{k_6} \tag{2.18}$$

If the three reversible reactions are assumed to be elementary reactions and they all follow a second order reaction kinetics, the following kinetic equations are obtained:

$$\frac{d[TG]}{dt} = -k_1[TG][MeOH] + k_2[DG][FAME]$$

$$\frac{d[DG]}{dt} = k_1[TG][MeOH] - k_2[DG][FAME] - k_3[DG][MeOH] + k_4[MG][FAME]$$

$$\frac{d[MG]}{dt} = k_3[DG][MeOH] - k_4[MG][FAME] - k_5[MG][MeOH] + k_6[GL][FAME]$$

$$\frac{d[FAME]}{dt} = k_1[TG][MeOH] - k_2[DG][FAME] + k_3[DG][MeOH]$$
$$- k_4[MG][FAME] + k_5[MG][MeOH] - k_6[GL][FAME] \tag{2.19}$$

$$\frac{d[MeOH]}{dt} = -k_1[TG][MeOH] + k_2[DG][FAME] - k_3[DG][MeOH]$$
$$+ k_4[MG][FAME] - k_5[MG][MeOH] + k_6[GL][FAME]$$

$$\frac{d[GL]}{dt} = k_5[MG][MeOH] - k_6[GL][FAME]$$

This differential equation system must be solved in order to obtain the different specific reaction rate constants and verify the accuracy of the model proposed. The complexity of the problem must be solved by the use of numerical methods.

The concentration evolution of the mono-, di-, triglycerides and methyl esters was obtained from the chromatographic analysis and recalculated by the volume of methanol added.

The initial concentrations of the mono-, di- and triglycerides (0.0023, 0.043 and 0.963 mol/lt respectively) were re-calculated after the addition of the corresponding methanol amount while the initial concentrations of glycerol and methyl esters were zero.

The concentrations of methanol and glycerol along the reaction were calculated through a material balance for the reaction where:

$$[GL] = ([TG]_0 - [TG]) + ([DG]_0 - [DG]) + ([MG]_0 - [MG]) \tag{2.20}$$

$$[MeOH] = [MeOH]_0 - ([FAME] - [FAME]_0) \tag{2.21}$$

2.4.3.3.2.   NUMERICAL MODELLING

Two different computing programs (GEPASI 3.30 and POLYMATH 6.0) were used to solve the problem.

To solve the present problem, the program GEPASI was provided with the mechanism described in equation 2.17, initial concentrations of all reactants, concentration evolution with time of all reactants and initial estimations for the ranges of the forward reaction rate constants. The equilibrium relation between the forward and reverse rate constants (equation 2.18) was also provided.

The program output included the optimal values for the specific rate constants (those that derived in the minimum value of sums of squares of deviations) and the theoretical concentration evolution for the different reactants calculated by the integration of the model (equation system 2.19).

The program POLYMATH 6.0 was then use to verify the concordance between the experimental data and the proposed model, using the specific reaction rate constants obtained from GEPASI.

2.4.3.3.3.   KINETIC RESULTS

The reaction kinetics was evaluated for the two different reaction conditions previously compared in figures 2.15 and 2.16.

For the reaction conducted at 60ºC, using a methanol to oil molar ratio of 6:1 and 1 % NaOH as catalyst, the initial data provided to program GEPASI were the initial concentration for all the reactants, the equilibrium rate constants and the estimated forward rate constants, detailed in table 2.11.

The provided estimated values for the forward rate constants were obtained from the numerical integration of equations 2.22 to 2.24.

$$k_1 = \frac{d[TG]/dt}{\left(\dfrac{[DG][FAME]}{K_{12}} - [TG][MeOH]\right)} \tag{2.22}$$

$$k_3 = \frac{d[DG]/dt - \left(k_1[TG][MeOH] - k_2[DG][FAME]\right)}{\left(\dfrac{[MG][FAME]}{K_{34}} - [DG][MeOH]\right)} \tag{2.23}$$

$$k_5 = \frac{d[MG]/dt - \left(k_3[DG][MeOH] - k_4[MG][FAME]\right)}{\left(\dfrac{[GL][FAME]}{K_{56}} - [MG][MeOH]\right)} \tag{2.24}$$

Experimental data for the concentration evolution of all the reactants was provided to the program together with the suggested ranges for k_1, k_3 and k_5. The results obtained are detailed in table 2.12.

Table 2.11. Initial conditions for the reaction conducted at 60ºC using 6:1 methanol molar ration and 1 % NaOH as catalyst.

Parameter	Units	Unrefined Oil	Pre-treated Oil
$[TG]_0$	mol/l	0.74	0.72
$[DG]_0$	mol/l	0.033	0.063
$[MG]_0$	mol/l	0.0018	0.0024
$[FAME]_0$	mol/l	0.0	0.16
$[MeOH]_0$	mol/l	4.44	4.3
$[GL]_0$	mol/l	0.0	0.0
K_{12}	-	0.5	0.08
K_{34}	-	0.46	14.7
K_{56}	-	25.2	15.7
k_1	l/mol.min	0.32	0.25
k_3	l/mol.min	0.91	1.63
k_5	l/mol.min	16.8	16.3

Table 2.12. Results provided by GEPASI for the reaction conducted at 60ºC using 6:1 methanol molar ration and 1 % NaOH as catalyst.

Parameter	Units	Unrefined Oil	Pre-treated Oil
$[TG]_{final}$	mol/l	0.062	0.045
$[DG]_{final}$	mol/l	0.038	0.0032
$[MG]_{final}$	mol/l	0.021	0.045
$[FAME]_{final}$	mol/l	2.01	2.25
$[MeOH]_{final}$	mol/l	2.43	2.21
$[GL]_{final}$	mol/l	0.65	0.69
SSD	-	0.004	0.049
k_1	l/mol.min	0.32	0.0205
k_3	l/mol.min	0.91	1.59
k_5	l/mol.min	16.8	16.32
k_2	l/mol.min	0.63	0.26
k_4	l/mol.min	1.96	0.11
k_6	l/mol.min	0.66	1.04

Where SSD is the sum of square deviations

Figures 2.17 and 2.18 show the comparison of the theoretical concentration evolution calculated for the model and the experimental data.
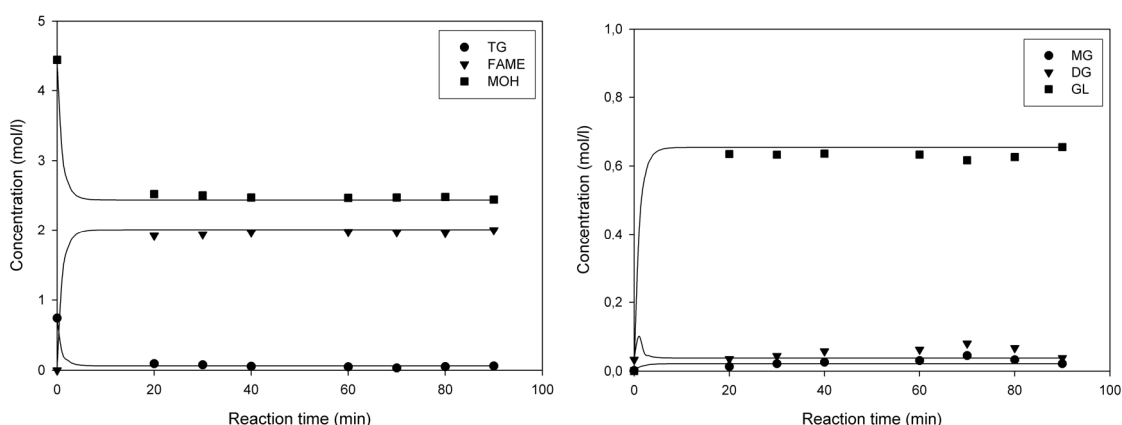
Figure 2.17. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1 % NaOH as catalyst, using unrefined *Cynara cardunculus* oil.
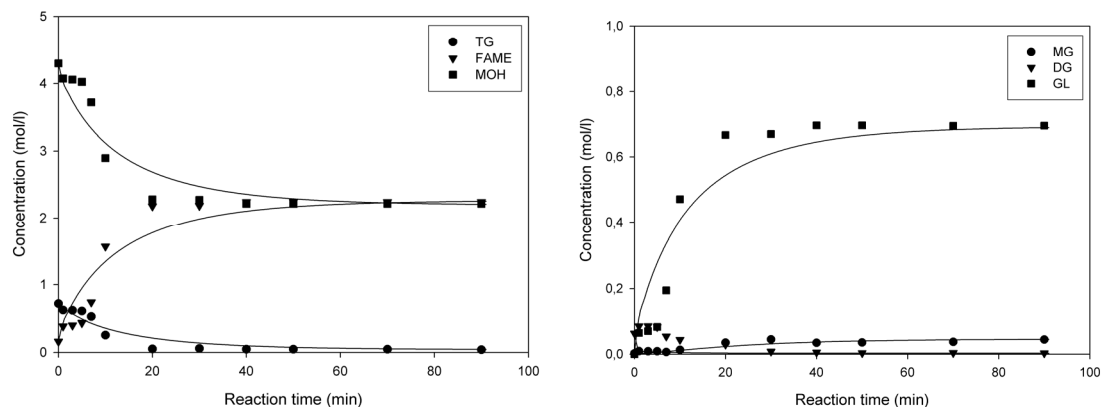
Figure 2.18. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1 % NaOH as catalyst, using pre-treated *Cynara cardunculus* oil.

For the reaction conducted at 60ºC, using a methanol to oil molar ratio of 6:1 and 1.5 % KOH as catalyst, the initial data provided to program GEPASI were the initial concentration for all the reactants, the equilibrium constants and the estimated forward rate constants, detailed in table 2.13.

Table 2.13. Initial conditions for the reaction conducted at 60ºC using 6:1 methanol molar ration and 1.5 % KOH as catalyst.

Parameter	Units	Unrefined Oil	Pre-treated Oil
$[TG]_0$	mol/l	0.74	0.72
$[DG]_0$	mol/l	0.033	0.063
$[MG]_0$	mol/l	0.0018	0.0024
$[FAME]_0$	mol/l	0.0	0.16
$[MeOH]_0$	mol/l	4.44	4.3
$[GL]_0$	mol/l	0.0	0.0
K_{12}	-	0.26	0.053
K_{34}	-	0.38	1.49
K_{56}	-	16.5	110
k_1	l/mol.min	0.30	0.29
k_3	l/mol.min	1.23	1.07
k_5	l/mol.min	22.6	9.35

Experimental data for the concentration evolution of all the reactants was provided to the program together with the suggested ranges for k_1, k_3 and k_5. The results obtained are detailed in table 2.14.

Table 2.14. Results provided by GEPASI for the reaction conducted at 60ºC using 6:1 methanol molar ration and 1.5 % KOH as catalyst.

Parameter	Units	Unrefined Oil	Pre-treated Oil
$[TG]_{final}$	mol/l	0.12	0.074
$[DG]_{final}$	mol/l	0.047	0.004
$[MG]_{final}$	mol/l	0.026	0.006
$[FAME]_{final}$	mol/l	1.80	2.20
$[MeOH]_{final}$	mol/l	2.64	2.26
$[GL]_{final}$	mol/l	0.58	0.7
SSD	-	0.0013	0.03
k_1	l/mol.min	0.30	0.039
k_3	l/mol.min	1.23	0.93
k_5	l/mol.min	22.6	9.35
k_2	l/mol.min	1.13	0.73
k_4	l/mol.min	3.28	0.63
k_6	l/mol.min	1.45	0.085

Where SSD is the sum of square deviations

Figures 2.19 and 2.20 show the comparison of the theoretical concentration evolution calculated for the model and the experimental data.
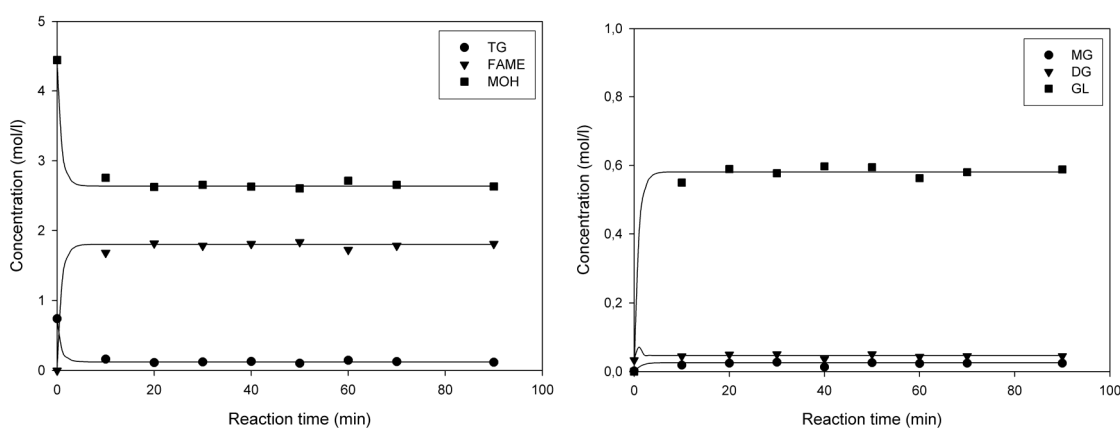
Figure 2.19. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1.5 % KOH as catalyst, using unrefined *Cynara cardunculus* oil.
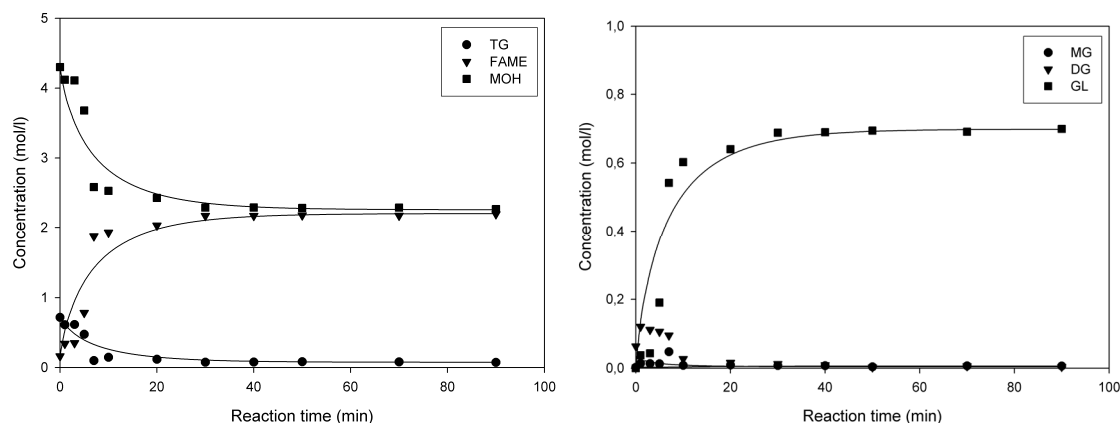
Figure 2.20. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1.5 % KOH as catalyst, using pre-treated *Cynara cardunculus* oil.

2.4.3.3.4. EFFECT OF TEMPERATURE

The influence of temperature on the specific reaction rate constants was determined from the logarithmic form of the integrated Arrhenius equation (equation 2.15).

For the reaction conducted at 25 and 40ºC, using a 6:1 methanol to oil molar ratio and 1 % NaOH, the concentration evolution and the kinetic modelling curves are shown in figures 2.21 and 2.22 The specific rate constants for these conditions are detailed in table 2.15. The influence of temperature for this reaction can be evaluated by comparing these results with those obtained for the same reaction conducted at 60ºC (table 2.12 and figure 2.18). The effect of temperature on the specific rate constants is shown in figure 2.23. Table 2.16 presents the results for A and E_a, for the different rate constants.

The results indicate that the forward and reverse rate constants for the first reaction, k_1 and k_2, decreased with temperature, while the rate constants for the rest of reaction increased. The highest activation energy values were found for the forward rate constants k_3 and k_5, resulting in an increase in the global reaction rate.
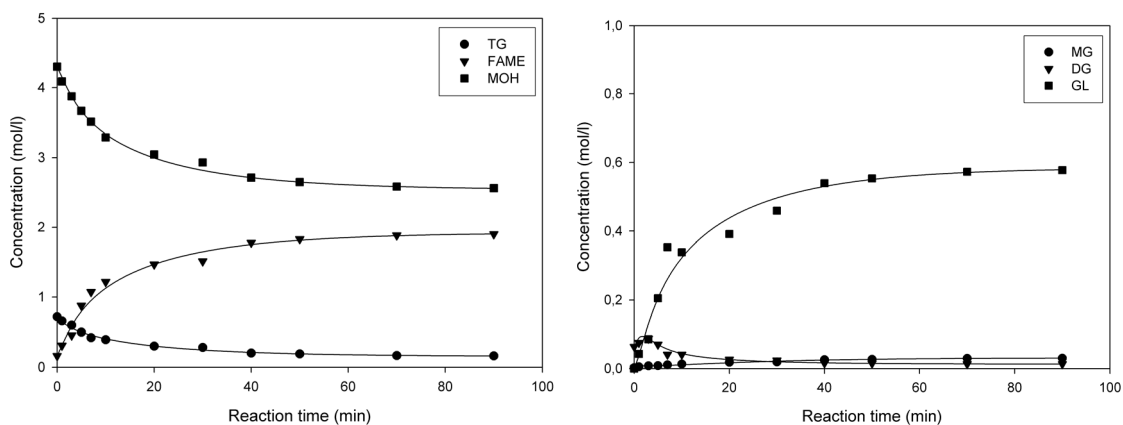
Figure 2.21. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 25ºC using 6:1 methanol molar ratio and 1 % NaOH as catalyst, using pre-treated *Cynara cardunculus* oil.
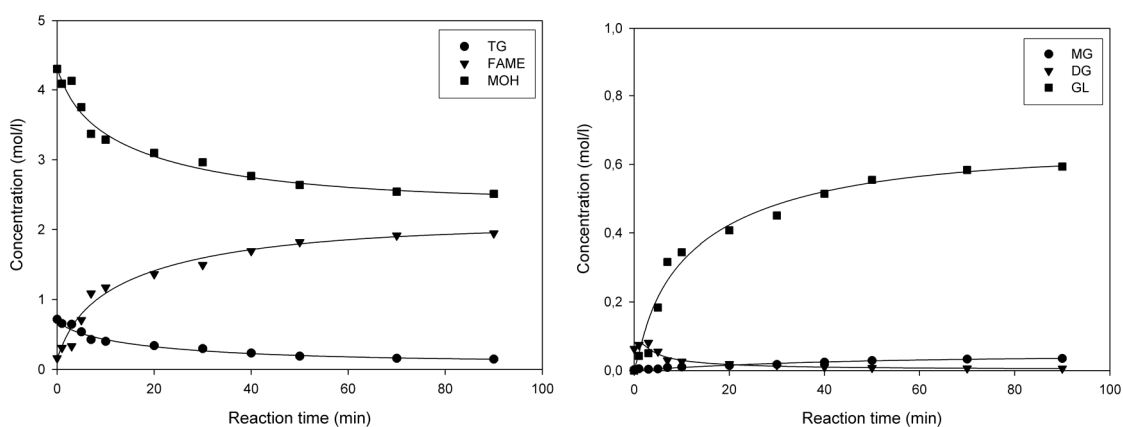
Figure 2.22. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 6:1 methanol molar ratio and 1 % NaOH as catalyst, using pre-treated *Cynara cardunculus* oil.

Table 2.15. Kinetic parameters for the reaction with 6:1 MeOH ratio and 1% NaOH.

	25ºC	40ºC	60ºC
k_1	0.027	0.030	0.021
k_2	0.44	0.92	0.26
k_3	0.13	0.16	1.59
k_4	0.068	0.023	0.11
k_5	1.65	4.16	16.32
k_6	0.12	0.32	1.04
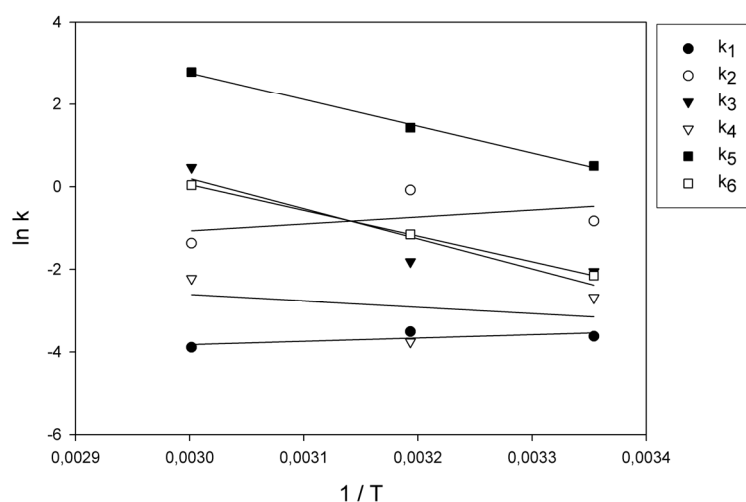
Figure 2.23. Effect of temperature on the reaction rate constants for the reaction conducted using 6:1 methanol molar ratio and 1 % NaOH as catalyst

Table 2.16. Arrhenius parameters for the reaction conducted using 6:1 methanol molar ratio and 1 % NaOH as catalyst

Specific rate constant	A (l/mol.min)	E_a (J/mol)
k_1	2.06×10^{-3}	-95
k_2	2.21×10^{-3}	-202
k_3	$3.91 \times 10^{+9}$	877
k_4	7.08	183
k_5	$4.95 \times 10^{+9}$	784
k_6	$1.32 \times 10^{+8}$	748

For the reaction conducted at 25ºC, using a 6:1 methanol to oil molar ratio and 1.5 % KOH, the concentration evolution and the kinetic modelling curves are shown in figure 2.24. The specific rate constants for these conditions are detailed in table 2.17. The influence of temperature for this reaction can be evaluated by comparing these results with those obtained for the same reaction conducted at 60ºC (table 2.14 and figure 2.20). The effect of temperature on the specific rate constants is shown in figure 2.25. Table 2.18 presents the results for A and E_a, for the different rate constants.
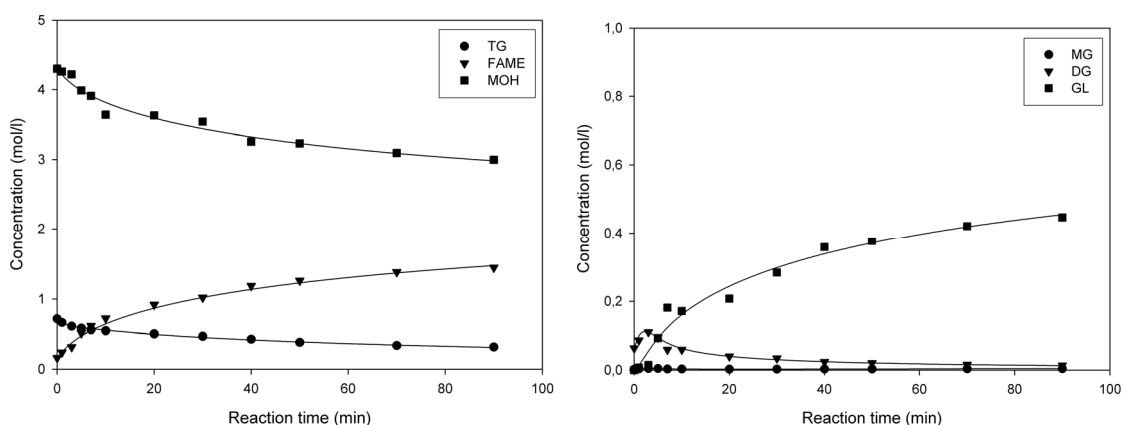
Figure 2.24. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 25ºC using 6:1 methanol molar ratio and 1.5 % KOH as catalyst, using pre-treated *Cynara cardunculus* oil.

Table 2.17. Kinetic parameters for the reaction with 6:1 MeOH ratio and 1.5% KOH.

	25ºC	60ºC
k_1	0.025	0.039
k_2	1.10	0.73
k_3	0.046	0.93
k_4	0.023	0.63
k_5	1.06	9.35
k_6	0.018	0.085
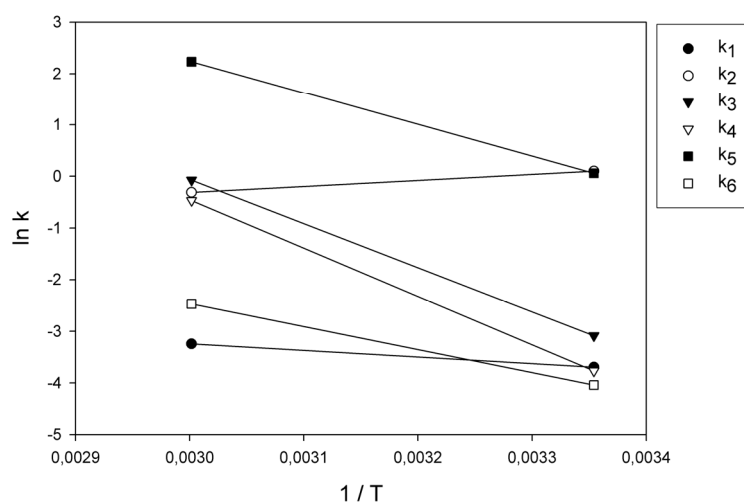
Figure 2.25. Effect of temperature on the reaction rate constants for the reaction conducted using 6:1 methanol molar ratio and 1.5 % KOH as catalyst

Table 2.18. Arrhenius parameters for the reaction conducted using 6:1 methanol molar ratio and 1.5 % KOH as catalyst

Specific rate constant	A (l/mol.min)	E_a (J/mol)
k_1	1.83	154
k_2	2.28×10^{-2}	-139
k_3	$1.32 \times 10^{+11}$	1029
k_4	$9.92 \times 10^{+11}$	1126
k_5	$1.06 \times 10^{+9}$	743
k_6	$5.56 \times 10^{+4}$	537

The results indicate that only the reverse rate constants for the first reaction, k_2, decreased with temperature, while the rate constants for the rest of reactions increased. The highest activation energy values were found for the forward and reverse rate constants for the second reaction, k_3 and k_4.

2.4.4. CONCLUSIONS

It was demonstrated that the ester yield is increased by the use of the pre-treatment steps. The best operation conditions were obtained for the reactions conducted at 60ºC, using twice the stoichiometric amount of methanol (6:1 molar ratio) and 1 % NaOH or 1.5 % KOH as catalyst. The increment in the ester yield is not only due to the esterification of the FFA, but also to the better use of the catalyst and the lack of soaps and emulsions.

The economy of the process is benefited by the additional ester amount obtained from the preesterification step. In addition, phase separation is easier for pre-treated oil transesterification, while the purification of both phases (biodiesel and glycerol) is simpler.

The choice of the same temperature (60ºC) for the degumming, preesterification and transesterification steps simplifies the whole process operation and allows the implementation of a semi-continuous process with three similar cycles of reaction - phase separation – evaporation.

The experience acquired during the optimisation of the different steps allows the implementation of a flexible system that can be employed for the production of biodiesel from *Cynara cardunculus* oil. However, this results could be adapted to other raw materials that present a high initial FFA content.

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3. BIODIESEL-DIESEL MIXTURES AND THEIR PROPERTIES
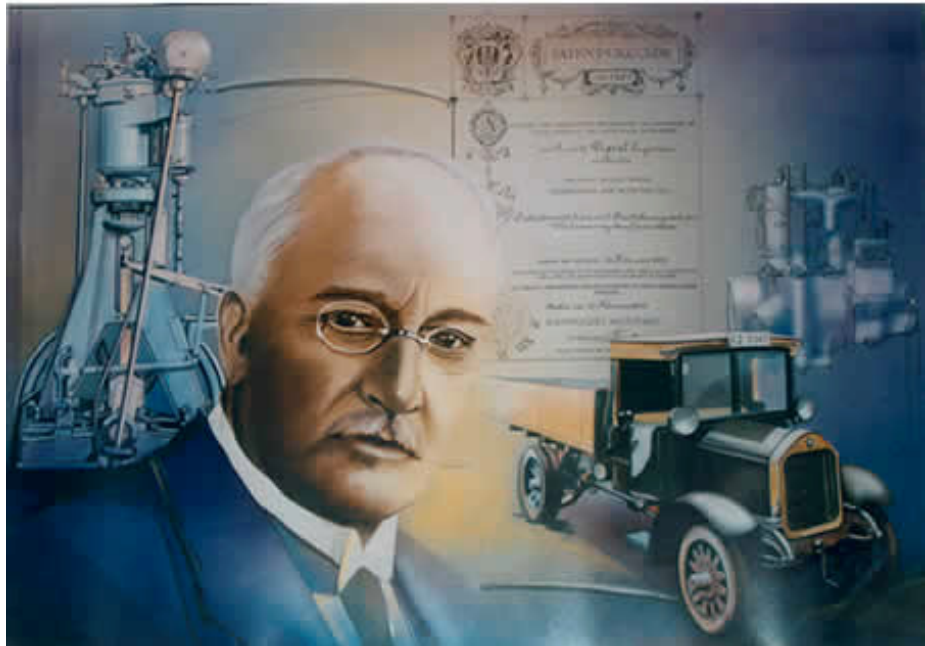
(RUDOLF DIESEL, 1858-1913)

"The diesel engine can be fed with vegetable oils and would help considerably in the development of agriculture of the countries which use it"

(RUDOLF DIESEL, 1911)

"The use of vegetable oils for engine fuels may seem insignificant today, but such oils may become, in the course of time, as important as petroleum and the coal-tar products of the present time...

Motive power can still be produced from the heat of the sun, always available, even when the natural stores of solid and liquid fuels are completely exhausted"

(RUDOLF DIESEL, 1912)

3.1. INTRODUCTION

The determination of fuel quality is an issue of great importance to the successful commercialisation of biodiesel. The chemical composition of biodiesel and diesel fuel is different, leading to differences in the physical and chemical properties. Diesel fuel is oxygen free and contains 30-35% aromatic hydrocarbons, 65-70% paraffins and traces of olefins, being most of its components in the C_{10} to C_{16} range. Biodiesel contains fatty acid alkyl esters mostly in the C_{16} to C_{18} range, with an oxygen content of 11% (w/w) *(Mittelbach 2004)*.

European standards for diesel fuel quality are contained in EN 590, however different quality parameters and test methods are needed for biodiesel.

Contaminants in biodiesel can lead to operational problems, including engine deposits, filter clogging, or fuel deterioration. Several standards have been developed to limit the level of contaminants, such as the EN 14214 and EN 14213 in Europe, and the ASTM D6751 in the United States. Virtually al methods used in the analysis of biodiesel are suitable for all biodiesel feedstock *(Knothe 2005)*.

Biodiesel can be blended in any proportion with fossil derived diesel fuel. Though most of the biodiesel nowadays produced is being commercialised in mixtures with diesel fuel, there is no specific standard for these mixtures.

The objective of this chapter is to analyse the application of diesel fuel normative (EN 590) to the mixtures of biodiesel and diesel fuel at different proportions, and determine the need of test methods and requirements specific to the mixtures.

3.2. BIODIESEL PROPERTIES AND ANALYTICAL METHODS

3.2.1. REQUIREMENTS AND TEST METHODS

Biodiesel quality parameters for its use as automotive fuel, are specified in Europe by EN 14214. The requirements and test methods are detailed in table 3.1.

Table 3.1. Biodiesel requirements and standard test methods in Europe *(EN 14214)*

Property	Unit	Minimum	Maximum	Test method
Ester content	% (w/w)	96,5	-	EN 14103
Density (15ºC)	kg/m^3	860	900	EN ISO 3675
Viscosity (40ºC)	mm^2/s	3.50	5.00	EN ISO 3104
Flash point	ºC	120	-	EN ISO 3679
Sulphur content	mg/kg	-	10.0	EN ISO 20846 EN ISO 20884
Conradson carbon residue	% (w/w)	-	0.30	EN ISO 10370
Cetane number	-	51.0	-	EN ISO 5165
Sulfated ash content	% (w/w)	-	0.02	ISO 3987
Water content	mg/kg	-	500	EN ISO 12937
Total contamination	mg/kg	-	24.0	EN 12662
Copper strip corrosion	-	Class 1		EN ISO 2160
Oxidative stability	h (110ºC)	6.00	-	EN 14112
Acid value	mg KOH/g	-	0.50	EN 14104
Iodine number	g I$_2$/100 g	-	120	EN 14111
Linolenic acid methyl ester	% (w/w)	-	12.0	EN 14103
Polyunsaturated methyl esters	% (w/w)	-	1.00	EN 14103
Methanol content	% (w/w)	-	0.20	EN 14110
Monoglycerides content	% (w/w)	-	0.80	EN 14105
Diglycerides content	% (w/w)	-	0.20	EN 14105
Triglycerides content	% (w/w)	-	0.20	EN 14105
Free glycerol	% (w/w)	-	0.02	EN 14105 EN 14106
Total glycerol	% (w/w)	-	0.25	EN 14105
Group I metals (Na + K)	mg/kg	-	5.00	EN 14108 EN 14109
Group II metals (Ca + Mg)	mg/kg	-	5.00	EN 14538
Phosphorus content	mg/kg	-	10.0	EN 14107
Cold filter plugging point Summer Winter	ºC	- -	0 -15	EN 116

3.2.2. ESTER CONTENT *(EN 14103)*

The methyl esters content in biodiesel is measured using capillary gas chromatography on polar stationary phases. The methyl esters are separated according to their chain length and the number of double bonds. The standard test method (EN 14103) considers the esters contained between the myristate (C14:0) and the nervonic acid methyl ester (C24:1), using methyl heptadecanoate as internal standard. A low ester content indicates incorrect reaction conditions, or the presence of contaminants such as unsaponifiable matter, residual alcohol, unreacted glycerides or glycerol.

The fatty acid composition of the original oil is usually determined under the same method and conditions, after its transesterification (EN 5508 and EN 5509). Fatty acid methyl ester standard mixtures are used for the identification of the different esters. The linolenic acid methyl ester content is also determined by this method. This unsaturated fatty acid is limited by specifications, as it can be a cause of low oxidative stability, and engine deposits.

Most chromatographic analysis are usually applied to methyl esters, but they will probably need modifications for the analysis or ethyl, isopropyl or higher alkyl esters *(Knothe 2005).*

3.2.3. DENSITY *(EN ISO 3675)*

The density of biodiesel is measured using calibrated areometers that are introduced into the sample previously acclimated to the specified temperature. The density of biodiesel depends mainly on its composition (chain length and double bonds) and its purity (presence of contaminants, such as methanol). Longer ester chains or contaminants, such as methanol, decrease the density, while the number of double bonds increases its value.

3.2.4. VISCOSITY *(EN ISO 3104)*

The kinematic viscosity of biodiesel is measured using calibrated Ostwald viscometers. An exact volume of the sample is introduced into the viscometer and acclimated to the specified temperature, usually into a controlled bath. The sample is dragged through a capillary tube until it exceeds the upper measuring mark. A chronometer is used to measure the time that the sample needs to pass from the upper to the lower mark. The kinematic

viscosity is calculated as a product of the time and the viscometer constant. The dynamic viscosity is calculated as the product of the kinematic viscosity and the density of the sample, both measured at the same temperature.

The viscosity of biodiesel increases with the chain length and the degree of saturation. A high viscosity can cause engine deposits as it affects the atomisation of the fuel upon injection.

The viscosity measurements can be used to follow the transesterification reaction kinetics, because during the reaction the viscosity of the original oil is reduced in almost one order of magnitude. The viscometric method measures the viscosity along the transesterification reaction, being a suitable and rapid method for process control purposes, that is in good agreement with GC analysis.

3.2.5. FLASH POINT *(EN ISO 3679)*

The flash point is the lowest temperature at which the vapour released by a liquid, can form an ignitable mixture with air. The flash point of biodiesel is measured using a Pensky-Martens closed cup equipment. This apparatus contains a small cup where the liquid sample is introduced and kept under continuous agitation. The cup is gradually heated while an open flame is directed into the cup at regular temperature intervals. When the contents of the cup ignite, the flash point temperature has been reached.

The flash point of a fuel is a security parameter, with great importance for its transport and storage. The flash point of biodiesel is one of its main advantages when compared to diesel fuel, as it is usually high, sometimes reaching 170ºC. Low flash point temperatures indicate the presence of residual alcohol.

3.2.6. SULPHUR CONTENT *(EN ISO 20846, EN ISO 20884)*

The sulphur content of biodiesel is measured using ultraviolet fluorescence spectrometry or wavelength-dispersive X-ray fluorescence spectrometry. The sulphur content of biodiesel is usually close to zero, specially when it is produced from fresh oils. However, small

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amounts of sulphur are detected when biodiesel is produced from waste oils, animal fats, or using acid catalysts.

3.2.7. CONRADSON CARBON RESIDUE *(EN ISO 10370)*

The carbon residue is the amount of carbonaceous matter left after evaporation and pyrolysis of a fuel sample under specific conditions. It measures the tendency of a fuel to produce deposits on injector tips and inside the combustion chamber. The carbon residue is a very important parameter for biodiesel quality as it is an indicator of rests of unreacted glycerides, FFA, soaps, catalyst residues, polyunsaturated FAME and polymers.

3.2.8. CETANE NUMBER *(EN ISO 5165)*

The cetane number is a dimensionless parameter used to measure the ignition quality of a diesel engine fuel. A higher cetane number indicates a shorter delay between the fuel injection and the ignition. Thus, it ensures a good cold start behaviour, a silent running and a smooth run of the engine. A lower cetane number indicates an incomplete combustion and causes engine knocking, increased gaseous and particulate exhaust emissions and high engine deposits. Peroxide compounds can be used as additives to increase the cetane number of a fuel. The cetane number of fatty acid alkyl esters increases with the length of the fatty acid chain and the length of the ester group and decreases with the number of double bonds. This represents an advantage for palm oil and tallow, and for the use of long-chain alcohols. The cetane number increases with time as some products formed during the oxidation, are considered as cetane improvers. *(Mittelbach 2004)*

To determine the cetane number of a fuel, a standard test engine is used. However, only a few laboratories are able to possess the standard engine because of its high price. The ignition performance is compared for the fuel sample and some reference fuels with known values. Reference cetane numbers of 100 and 15 are assigned to hexadecane ($C_{16}H_{34}$, known as cetane) and 2,2,4,4,6,8,8-heptamethylnonane ($C_{16}H_{34}$, known as HMN) respectively *(Mittelbach 2004).*

3.2.9. SULPHATED ASH CONTENT *(EN ISO 3987)*

The ash content is measured by burning the fuel and calcining it at a high temperature (775ºC), causing loss of moisture, oxidation and the decomposition of carbonates and other compounds. For biodiesel ash content, concentrated sulphuric acid is added to the residue after combustion, in order to transfer metallic impurities into the corresponding sulphates. In this way, catalyst residues in the form of sodium and potassium sulphates, can also be measured, because they are less volatile than their corresponding oxides. Excessive ash can lead to engine deposits and high abrasive wear levels. Sulphated ash content in biodiesel is an indicator of residual catalyst.

3.2.10. WATER CONTENT *(EN ISO 12937)*

Biodiesel is insoluble in water but it may contain small amounts of water dissolved or as suspended droplets. Water is also absorbed during storage, as fatty acid methyl esters are hygroscopic. Water is a problem for fuel injection equipment because it contributes to corrosion. Contact with water also causes an increase in the acid value and in the microbial growth *(Knothe 2005, Mittelbach 2004)*.

The water content in biodiesel is measured using a coulometric Karl Fisher titration apparatus. Inside the titration cell, the presence of water induces the generation of iodine, by electrochemical oxidation of iodide, and a reaction between I_2 and SO_2 occurs until unreacted iodine is detected. Faraday's law is used to calculate the amount of iodine generated from the quantity of electricity required. The water content is proportional to the iodine generated.

3.2.11. TOTAL CONTAMINATION *(EN 12662)*

The total contamination of a fuel measures the insoluble material retained after its filtration. The fuel is previously heated at 40ºC, and a standard 0.8 μm cellulose filter is used. A high level of insoluble impurities causes blockages of fuel filters and injection pumps. Total contamination in biodiesel is mainly caused by the presence of soaps and sediments. Distillation of the fuel can be used to reduce its total contamination *(Mittelbach 2004)*.

3.2.12. COPPER STRIP CORROSION *(EN ISO 2160)*

The copper strip corrosion is a measure of the tendency of a fuel to cause corrosion to copper, zinc and bronze parts of the engine and the storage tank. To determine this parameter a copper strip is submerged into the fuel at 50ºC during 3 hrs. It is then washed and its colour is compared to standards with different corrosion levels. Corrosion in biodiesel is mainly caused by sulphur components and FFA *(Mittelbach 2004).*

3.2.13. OXIDATIVE STABILITY *(EN 14112)*

Storage stability is the ability of a fuel to resist chemical changes during long term storage. Contact with air is the mayor cause of oxidation and causes an increase in the acid value and the viscosity of biodiesel. It also darkens biodiesel colour and causes a bad smell. Natural antioxidants are contained in the original oils, but some of them are removed during oil refining steps, so antioxidant additives are recommended for biodiesel. The number of double bonds in biodiesel are also a cause of autoxidation *(Knothe 2005).*

The oxidative stability of biodiesel is measured using a Rancimat apparatus, where oxidation conditions are accelerated by passing an air flow at 110ºC through a cell containing the sample. The air sweeps the oxidation vapours to a water reservoir where the conductivity changes with time are measured. The method establishes as the final time, the moment when there is a sudden increase in conductivity (induction period, in hrs).

3.2.14. ACID VALUE *(EN 14104)*

The acid value is the measure of the amount of KOH needed to neutralise the mineral acids and the FFA present in 1 g of biodiesel, and is indicated as mgKOH/g. To determine the acid value, a weighted sample is diluted in an organic solvent (iso-propanol or a mixture of toluene and ethanol), and titrated with ethanolic KOH solution.

The FFA content in biodiesel depends on the original feedstock and the oil refining steps, but can also be caused by the use of acid catalysts and the presence of water after long time storage. High acid values can cause corrosion and engine deposits.

3.2.15. Iodine number *(EN 14111)*

The iodine value measures the total unsaturation of an oil or its correspondent biodiesel as the amount of I_2 necessary to saturate 100 g of sample. It is expressed as $gI_2/100g$. To determine the iodine number, a weighted sample is diluted in a solvent (acetic acid and ciclohexane mixture), and reacted with Wijs reagent (containing iodine monochloride) during one hour in the dark. Samples are titrated with sodium thiosulphate solution after the addition of water and KI solution, using starch as indicator. Titration of the samples is then compared with a blank.

Table 3.2. Contribution factors for the different methyl esters, affecting the calculation of iodine number

Fatty acid	Contribution
C16:1	0.950
C18:1	0.860
C18:2	1.732
C18:3	2.616
C20:1	0.785
C22:1	0.723

The iodine number can also be calculated from the fatty acid composition of the oil or biodiesel. The value is calculated using the contribution factor of the unsaturated fatty acids, detailed in table 3.2, and their respective composition.

3.2.16. Methanol content *(EN 14110)*

Most of the alcohols used in the transesterification reaction are more soluble in the glycerol phase than in the ester phase and so they are removed with glycerol. However, after separation, almost 2-4% alcohol remains in the ester phase. Stripping processes and water washing are used to remove the rests of alcohol. The alcohol amount left in biodiesel is usually too low to cause problems on fuel performance but it severely affects flash point. With only 1% methanol content in biodiesel, the flash point is reduced from 170 to 40ºC, so the rests of methanol are important because they lower the flash point and represent a potential safety hazard *(Knothe 2005)*.

To determine the methanol content in biodiesel, the samples are heated at 80ºC in hermetically sealed vials in order to evaporate the methanol present. A volume of the gas

phase is analysed by capillary gas chromatography, using 2-propanol as internal standard. Methanol-free FAME reference samples are used to obtain a calibration curve.

3.2.17. MONO-, DI- AND TRIGLYCERIDES CONTENT *(EN 14105)*

EN 14105 is used for simultaneous determination of glycerol, mono-, di-, and triglycerids by capillary gas chromatography, with non-polar, high-temperature columns. A cool on-column injector must be used for correct triglycerides detection. Two internal standards are needed for calibrating and analysis: 1,2,4-butanetriol is used to determine and calibrate the glycerol content, and 1,2,3-tricaproyl glycerol (tricaprin) is used to determine and calibrate the mono-, di- and triglycerides contents. Prior to analysis, all the samples are derivatised with N-methyl-N-trimethylsilyl-trifluoracetamide (MSTFA) in order to improve the chromatographic properties of the glycerids analysed. References substances such as glycerol, monoolein, monopalmitin, monoestearin, diolen and triolein are used to obtain appropriate calibration curves.

The amount of unreacted glycerids depends on the production process, and can be reduced by using the optimal reaction conditions or distilling the final product *(Mittelbach 2004)*. High glycerides contents can cause injector deposits and increase the viscosity.

3.2.18. FREE AND TOTAL GLYCEROL (*(EN 14105, EN 14106)*

Most of the glycerol is separated from biodiesel by settling or centrifugation, but some glycerol may remain as suspended droplets or dissolved in the ester phase. The rests of alcohol increase the solubility of glycerol in biodiesel. Water washing steps, specially when using hot water, remove most of the glycerol dissolved or in droplets.

Free glycerol content in biodiesel can settle in storage tanks, creating a viscous mixture that can plug fuel filters and cause combustion problems in the engine.

HPLC and iodometric methods can be used to determine both free and total glycerol, but only GC methods are acceptable for demonstrating the conformity with standards *(Knothe 2005)*.

Free glycerol is determined by GC, according to EN 14105 (see 3.2.17). The total glycerol content is calculated from the contribution of the free glycerol and the measured amounts of mono-, di-, and triglycerides.

3.2.19. ALKALINE METALS *(EN 14108, EN 14109, EN 14538)*

Alkaline catalysts are more soluble in glycerol than in the ester phase. Thus, they are separated from biodiesel together with glycerol and during the washing steps, so only low amounts of them remain in the ester phase. The standards for alkaline metals and sulphated ash determine the maximum catalyst that can be present in biodiesel in order to avoid abrasive deposits in the engine *(Knothe 2005)*.

Sodium and potasium contents are determined by flame atomic absorption spectrometry, while magnesium and calcium are determined by ICP spectrometry.

3.2.20. PHOSPHORUS CONTENT *(EN 14107)*

The phosphorous content of biodiesel is measured using inductively coupled argon plasma emission spectrometry (ICP). The presence of phospholipids contained in the feedstock also increases the phosphorous content in biodiesel.

3.2.21. COLD FILTER PLUGGING POINT *(EN 116)*

The CFPP measures the lowest temperature at which 20 ml of a fuel pass through a filter in 60 seconds. The CFPP predicts the minimum operability temperature of a fuel *(Knothe 2005)*.

3.3. DIESEL FUEL PROPERTIES AND ANALYTICAL METHODS

3.3.1. REQUIREMENTS AND TEST METHODS

Diesel fuel quality parameters, are specified in Europe by EN 590. The requirements and test methods are detailed in table 3.3.

Table 3.3. Diesel fuel requirements and standard test methods in Europe *(EN 590)*

Property	Unit	Minimum	Maximum	Test method
Cetane number	-	51.0	-	EN ISO 5165
Cetane index	-	46.0	-	EN ISO 4264
Density (15ºC)	kg/m^3	820	845	EN ISO 3675
Polycyclic aromatic hydrocarbons	% (w/w)	-	11.0	EN 12916
Sulphur content	mg/kg	-	50	EN ISO 20846 EN ISO 20847 EN ISO 20884
Flash point	ºC	55	-	EN ISO 2719
Conradson carbon residue	% (w/w)	-	0.30	EN ISO 10370
Ash content	% (w/w)	-	0.01	EN ISO 6245
Water content	mg/kg	-	200	EN ISO 12937
Total contamination	mg/kg	-	24	EN 12662
Copper strip corrosion	-	Class 1		EN ISO 2160
Oxidative stability	g/m^3	-	25	EN ISO 12205
Lubricity	µm	-	460	ISO 12156-1
Viscosity (40ºC)	mm^2/s	2.00	4.50	EN ISO 3104
Distillation Recovered at 250ºC Recovered at 350ºC 95% (v/v) recovered at	% (v/v) ºC	- 85 -	65 - 360	EN ISO 3405
Cold filter plugging point Summer Winter	ºC	- -	0 -10	EN 116

3.4. PROPERTIES OF THE MIXTURES

3.4.1. SIMILAR PROPERTIES FOR BIODIESEL AND DIESEL FUEL

There are some properties that have similar requirements and test methods for both biodiesel and diesel fuel (table 3.4). In this case, the mixtures of them both can be analysed under the same methods and conditions than the original fuels, and they will have the same requirements.

Table 3.4. Biodiesel and diesel fuel properties with similar requirements and standard test methods in Europe *(EN 14214 and EN 590)*

Property	Test method	Limits
Cetane number	EN ISO 5165	> 51
Carbon residue	EN ISO 10370	< 0.3% (w/w)
Total contamination	EN ISO 12662	< 24 mg/kg
Copper strip corrosion	EN ISO 2160	Class 1

3.4.1.1. CETANE NUMBER *(EN ISO 5165)*

The cetane number requirements for both diesel and biodiesel are coincident and the cetane number for biodiesel is usually higher than the value for diesel. In the case of biodiesel, the cetane number is affected by its fatty acid alkyl esters composition (length of the chain and the ester part, and number of double bonds). Thus, it could be assumed that, if both diesel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the cetane number, then a mixture of them both at any proportion will satisfy the diesel normative too (EN 590). Although an appropriate correlation is needed to calculate the exact cetane number for the mixture, it can be estimated that it will be between the values of the separate components. However, experiments are needed to obtain a correlation to calculate the cetane number of a mixture, knowing the cetane numbers of the pure components, and the mixture composition. Such a correlation, will also be helpful to calculate the type and amount of biodiesel needed to increase the cetane number of a diesel with a lower value than required.

3.4.1.2. CARBON RESIDUE *(EN ISO 10370)*

The carbon residue requirements for both diesel and biodiesel are coincident. In the case of biodiesel, the carbon residue indicates the presence of impurities, such as rests of catalysts, soaps, FFA and unreacted glycerides, but in the case of diesel fuel, it is not an important quality parameter. As the carbon residue is measured as a mass percentage, the carbon residue content of a mixture can be calculated by a mass balance.

$$CR_M = CR_{DF} \cdot X_{DF} + CR_{BD} \cdot X_{BD} \qquad (3.1)$$

Where CR_i is the carbon residue content in the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.

Figure 3.1. Carbon residue of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

Thus, if both diesel fuel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the carbon residue content, then a mixture of them both at any proportion will satisfy the diesel normative too (EN 590).

3.4.1.3. TOTAL CONTAMINATION *(EN ISO 12662)*

The total contamination requirements for both diesel and biodiesel are coincident. As the total contamination is measured as a mass content, the total contamination of a mixture of diesel fuel and biodiesel can be calculated by a mass balance.

$$TC_M = TC_{DF} \cdot X_{DF} + TC_{BD} \cdot X_{BD} \qquad\qquad (3.2)$$

Where TC_i is the total contamination in the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.

Figure 3.2. Total contamination of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

Thus, if both diesel fuel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the total contamination, then a mixture of them both at any proportion will satisfy the diesel normative too (EN 590).

3.4.1.4. COPPER STRIP CORROSION *(EN ISO 2160)*

The copper strip corrosion requirements for both diesel and biodiesel are coincident. As the copper strip corrosion is determined by comparison to a certain colour standard, the copper strip corrosion of a mixture of diesel fuel and biodiesel can not be calculated. Thus, experiments are needed to verify the copper strip corrosion of the mixtures and to determine whether all the mixtures satisfy the diesel normative. However, as corrosion to copper is caused by some components present in the separated fuels, it can be assumed that this components will behave in the same way when both fuels are mixed. So, if both diesel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the copper strip corrosion, then a mixture of them both at any proportion will satisfy the diesel normative too (EN 590).

3.4.2. PROPERTIES WITH DIFFERENT LIMITS

There are some properties that have similar test methods for both biodiesel and diesel fuel, but the limit requirements are different (table 3.5). In this case, the mixtures of them both can be analysed under the same methods and conditions than the original fuels. However, as the requirements for biodiesel and diesel fuel are different, it is possible that mixtures with a high biodiesel content will not satisfy diesel fuel normative. Thus, there will be a maximum biodiesel percentage that can be added to diesel fuel in order to satisfy EN 590.

Table 3.5. Biodiesel and diesel fuel properties with similar standard test methods but different requirements *(EN 14214 and EN 590)*

Property	Test method	Diesel Limits	Biodiesel Limits
Density	EN ISO 3675	820-845 kg/m^3	860-900 kg/m^3
Ash content	EN ISO 6245	< 0.01% (w/w)	< 0.02% (w/w)
Water content	EN ISO 12937	< 200 mg/kg	< 500 mg/kg
Viscosity	EN ISO 3104	2.0-4.5 mm^2/s	3.5-5.0 mm^2/s
CFPP	EN 116	< 0ºC (summer) < -10ºC (winter)	< 0ºC (summer) < -15ºC (winter)

3.4.2.1. DENSITY *(EN ISO 3675)*

The density requirements for diesel and biodiesel are different, because biodiesel is usually denser than diesel fuel. An appropriate correlation is needed to calculate the density of the mixture, but it can be estimated that it will be between the values of the separate components. That correlation will calculate the density of the mixture, knowing the density of the pure fuels, and the mixture composition. It will also be helpful to calculate the maximum amount of biodiesel allowed in the mixture in order to satisfy the diesel fuel normative.

3.4.2.2. ASH CONTENT *(EN ISO 6245)*

The ash content requirements for diesel fuel and biodiesel are different. Although the test methods presents some differences, both are based on the same principles. The amount of ashes is measured as a mass content, so the ash content of a mixture of diesel fuel and biodiesel can be calculated by a mass balance:

$$AC_M = AC_{DF} \cdot X_{DF} + AC_{BD} \cdot X_{BD} \tag{3.3}$$

Where AC_i is the ash content of the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.
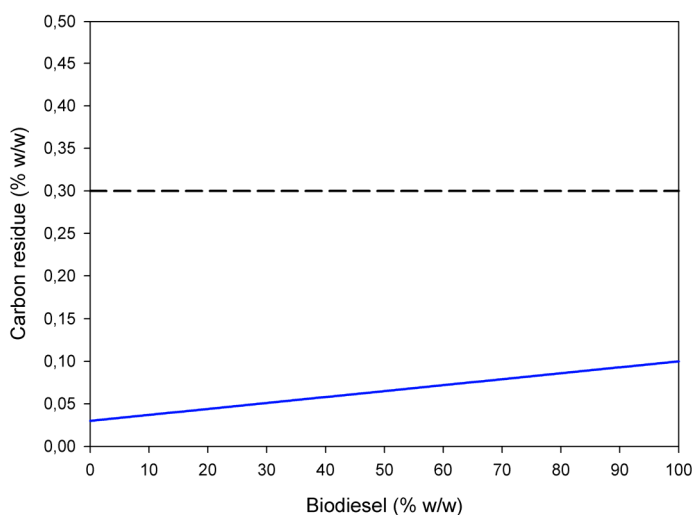
Even if both fuels satisfy their respective normative (EN 590 and EN 14214) with respect to the ash content, it is possible that some mixtures of them both will not satisfy the diesel normative (EN 590). Equation 3.3 can be helpful to calculate the maximum amount of biodiesel allowed in the mixture in order to satisfy the diesel fuel normative.

Figure 3.3. Ash content of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

3.4.2.3.   WATER CONTENT *(EN ISO 12937)*

Standard limits for water in biodiesel and diesel are different, because biodiesel usually contains more water than diesel. While biodiesel can contain up to 1500 ppm water, diesel usually reaches only 50 ppm. Water due to condensation may be present on the bottom of diesel storage tanks, so water content in biodiesel or diesel must be analysed frequently, specially during long term storage *(Knothe 2005)*.

The water content requirements for diesel fuel and biodiesel are different. The amount of water is measured as a mass content, so the water content of a mixture of diesel fuel and biodiesel can be calculated by a mass balance:

$$WC_M = WC_{DF} \cdot X_{DF} + WC_{BD} \cdot X_{BD} \qquad (3.4)$$

Where WC_i is the water content of the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.
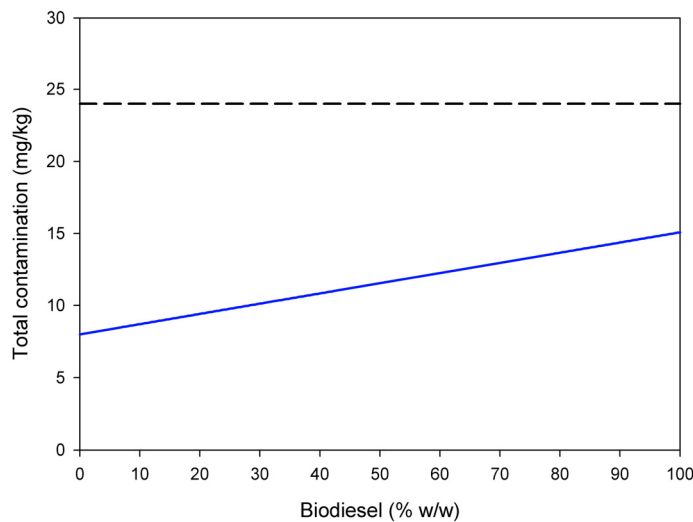

Figure 3.4. Water content of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

Even if both diesel fuel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the water content, it is possible that some mixtures of them both will not satisfy the diesel normative (EN 590). Equation 3.4 can be helpful to calculate the maximum amount of biodiesel allowed in the mixture in order to satisfy the diesel fuel normative. If diesel standards have to be met, biodiesel must be kept as dry as possible.

3.4.2.4. VISCOSITY *(EN ISO 3104)*

The Viscosity requirements for diesel and biodiesel are different, because biodiesel is usually more viscous than diesel fuel. An appropriate correlation is needed to calculate the viscosity of the mixture, but it can be estimated that it will be between the values of the separate components. That correlation will calculate the viscosity of the mixture, knowing the viscosity of the pure fuels, and the mixture composition. It will also be helpful to calculate the maximum amount of biodiesel allowed in the mixture in order to satisfy the diesel fuel normative.

There are different models to calculate the viscosity of mixtures, that can be found in bibliography:

➢ Model for mixtures *(Perry 1997)*.

$$\mu_{mixture} = \exp\left(\sum X_i \cdot \ln \mu_i\right) \tag{3.5}$$

where μ is the viscosity of the mixture or the pure components and X_i is the volume fraction of every component.

➢ Kendall and Monroe model *(Perry 1997)*.

$$\mu_{mixture} = \left(\sum X_i \cdot \sqrt[3]{\mu_i}\right)^3 \tag{3.6}$$

➢ Grunberg and Nissan model *(Perry 1984)*.

$$\mu_{mixture} = \exp\left(X_1 \cdot \ln \mu_1 + X_2 \cdot \ln \mu_2 + 2 \cdot X_1 \cdot X_2 \cdot \overline{G_{12}}\right) \tag{3.7}$$

Where, for every mixture:

$$G_{12} = \frac{\ln \mu_{mixture} - X_1 \cdot \ln \mu_1 - X_2 \cdot \ln \mu_2}{2 \cdot X_1 \cdot X_2} \tag{3.8}$$

and $\overline{G_{12}}$ is the average value for all calculated G_{12}.

In general, for polar mixtures the $\overline{G_{12}}$ value is of about -0.09, for non-polar mixtures the $\overline{G_{12}}$ value is of about -0.22 and for polar-non-polar mixtures it can be positive or negative, being a function of temperature in all the cases.

3.4.2.5. COLD FILTER PLUGGING POINT (CFPP) *(EN 116)*

The CFPP requirements for diesel and biodiesel are similar for summer use but are different for winter use. An appropriate correlation is needed to calculate the CFPP of the mixture, but it can be estimated that it will be between the values of the separate components. That correlation will calculate the CFPP of the mixture, knowing the CFPP of the pure fuels, and the mixture composition. It will also be helpful to calculate the maximum amount of biodiesel allowed in the mixture in order to satisfy the diesel fuel normative.

3.4.3. PROPERTIES WITH DIFFERENT TEST METHODS

There are some properties that have different test methods and requirements for biodiesel and diesel fuel (table 3.6). In this case, the mixtures of them both have to be analysed under the methods and conditions specified for diesel fuel. As the requirements for biodiesel and diesel fuel are different, it is possible that mixtures with a high biodiesel content will not satisfy diesel fuel normative. Thus, there will be a maximum biodiesel percentage that can be added to diesel fuel in order to satisfy EN 590.

Table 3.6. Biodiesel and diesel fuel properties with different requirements and standard test methods *(EN 14214 and EN 590)*

	Diesel		Biodiesel	
Property	**Test method**	**Limits**	**Test method**	**Limits**
Sulphur content	EN ISO 20884 EN ISO 20846 EN ISO 20847	< 50 mg/kg	EN ISO 20846 EN ISO 20884	< 10 mg/kg
Flash point	EN ISO 2719	> 55ºC	EN ISO 3679	> 120ºC
Oxidative stability	EN ISO 12205	< 25 g/cm^3	EN 14112	> 6 hrs at 110ºC

3.4.3.1. SULPHUR CONTENT *(EN ISO 20884, EN ISO 20846, EN ISO 20847)*

The sulphur content requirements for diesel fuel and biodiesel are different, because biodiesel usually present lower sulphur percentages than diesel fuel. Although the test methods presents some differences, both are based on the same principles. The amount of sulphur is measured as a mass content, so the sulphur content of a mixture of diesel fuel and biodiesel can be calculated by a mass balance:

$$SC_M = SC_{DF} \cdot X_{DF} + SC_{BD} \cdot X_{BD} \tag{3.9}$$

Where SC_i is the sulphur content of the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.

Thus, if both diesel fuel and biodiesel satisfy their respective normative (EN 590 and EN 14214) with respect to the sulphur content, then a mixture of them both at any proportion will satisfy the diesel normative too (EN 590). In addition, biodiesel can be used to decrease the sulphur content of a diesel fuel that exceeds the required limits. Equation 3.9

can be useful to calculate the minimum amount of biodiesel needed in the mixture in order to satisfy the diesel fuel normative.
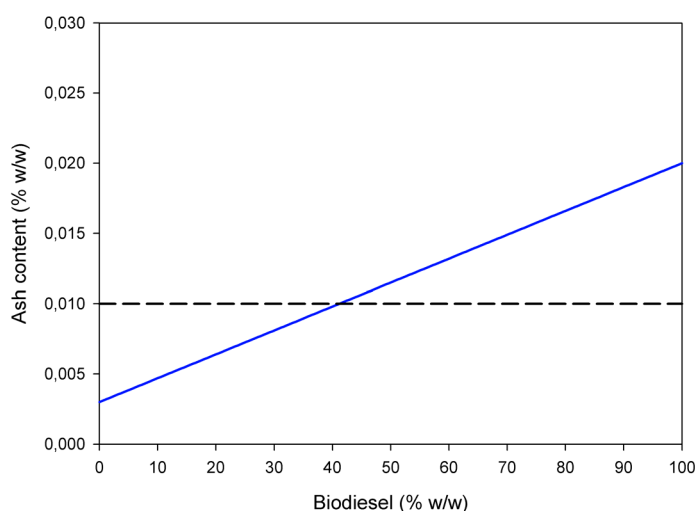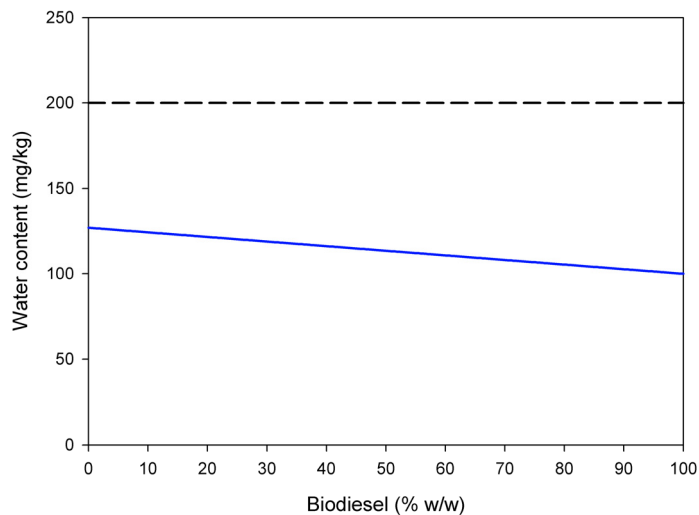

Figure 3.5. Sulphur content of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

3.4.3.2. FLASH POINT *(EN ISO 2719, EN ISO 3679)*

The flash point requirements for diesel fuel and biodiesel are different, because biodiesel usually presents higher flash point temperatures than diesel fuel. Although the test methods present some differences, both are based on the same principles. An appropriate correlation is needed to calculate the flash point of the mixture, but it can be estimated that it will be between the values of the separate components. That correlation will calculate the flash point of the mixture, knowing the flash of the pure fuels, and the mixture composition. As the most volatile components present in diesel fuel, will still be present in the mixtures, the flash point of the mixtures is expected to be closer to the flash point of the original diesel fuel.

3.4.3.3. OXIDATIVE STABILITY *(EN ISO 12205, EN ISO 14112)*

Although the oxidative stability test methods used for diesel fuel and biodiesel are different, both are based on the same principles: oxidation conditions are artificially accelerated for the samples, using high temperatures (95ºC for diesel fuel and 110ºC for

biodiesel) and oxidant gas flow (oxygen for diesel fuel and air for biodiesel). The main differences of the methods are related to the units of the parameters measured. The test method for diesel fuel measures the concentration (in g/m^3) of insoluble compounds generated after 16 hrs oxidation, while the test method for biodiesel measures the variation of conductivity with time, and establishes as the final time, the moment when there is a sudden increase in conductivity (induction period, in hrs).

As it is not possible to directly compare both parameters, there is no actual correlation for the oxidative stability of the biodiesel-diesel fuel mixtures. Methods for diesel fuel may not be suitable for biodiesel or biodiesel-diesel fuel blends, as the compounds generated during oxidation are different.

Experimental work is needed to determine whether there is any tendency in the oxidative stability of the mixtures, when they are analysed using the test method for diesel fuel.

3.4.4. PROPERTIES ONLY APPLIED TO DIESEL FUEL

There are some properties that are only included in diesel fuel specifications (table 3.7). In this case, the mixtures of biodiesel and diesel fuel have to be analysed under the methods and conditions specified for diesel fuel. It is possible that mixtures with a high biodiesel content will not satisfy diesel fuel normative. Thus, there will be a maximum biodiesel percentage that can be added to diesel fuel in order to satisfy EN 590.

Table 3.7. Diesel fuel properties that are not included in biodiesel standards in Europe *(EN 590)*

Property	Test method	Limits
Cetane index	EN ISO 4264	> 46
Polycyclic aromatic hydrocarbons	EN 12916	< 11% (w/w)
Lubricity	EN ISO 12156-1	< 460 μm
Distillation	EN ISO 3405	< 65% (v/v) at 250ºC > 85% (v/v) at 350ºC 95% (v/v) at < 360ºC

3.4.4.1. CETANE INDEX *(EN ISO 4264)*

The cetane index is used to predict the cetane number of a diesel fuel when the standard test motor is not available, or when the sample amount is not enough for the motor experiment. The cetane index of a diesel fuel is calculated using the following correlation:

$$CI = 454.74 - 1641.416 \cdot \delta + 774.74 \cdot \delta^2 - 0.554 \cdot T + 97.803 \cdot (\log T)^2 \qquad (3.10)$$

where δ is the density in gr/cm^3 of the sample, measured at 15ºC, and T is the temperature at which 50% (v/v) of the sample distilles.

This correlation is applicable to fuels obtained from direct distillation, catalytic cracking or their mixtures, but it is not useful for pure hydrocarbons, synthetic fuels, crude oil or products with a low final distillation temperature.

3.4.4.2. POLYCYCLIC AROMATIC HYDROCARBONS (PAH) *(EN ISO 12916)*

The PAH content in diesel fuel is measured by HPLC, using a polar column. The samples are diluted in heptane and injected into the chromatograph where the monoaromatic (MAH), diaromatic (DAH), and tri+-aromatic (T+AH) hydrocarbons are separated. The PAH are calculated as the sum of the DAH and the T+AH, while the total aromatic hydrocarbons are calculated as the sum of the MAH and the PAH. As the PAH content is measured as a mass percentage, the PAH content of a mixture of diesel fuel and biodiesel can be calculated by a mass balance.

$$PAH_M = PAH_{DF} \cdot X_{DF} + PAH_{BD} \cdot X_{BD} \qquad (3.11)$$

Where PAH_i is the PAH content in the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the mass fraction of diesel fuel or biodiesel in the mixture.

As biodiesel does not present PAH in its composition, the value for PAH_{BD} is zero, and the mixtures of biodiesel and diesel fuel, will present lower contents in PAH than the original diesel fuel, for any biodiesel proportion.

Thus, if diesel fuel satisfies its normative (EN 590) with respect to the PAH content, then a mixture of diesel fuel with biodiesel at any proportion will satisfy the diesel normative too.

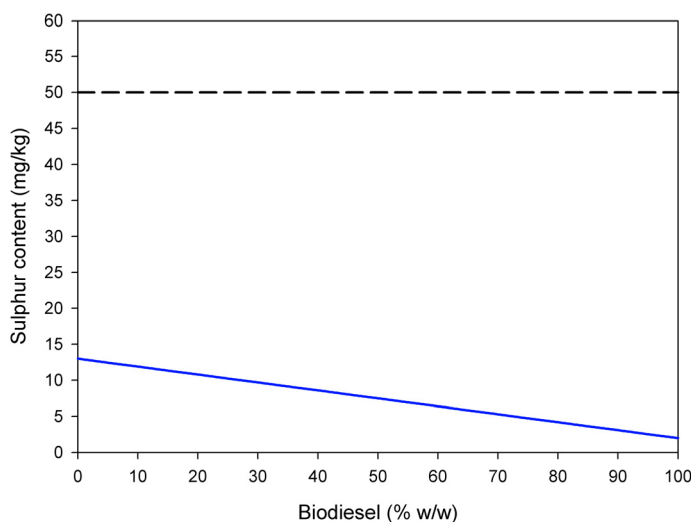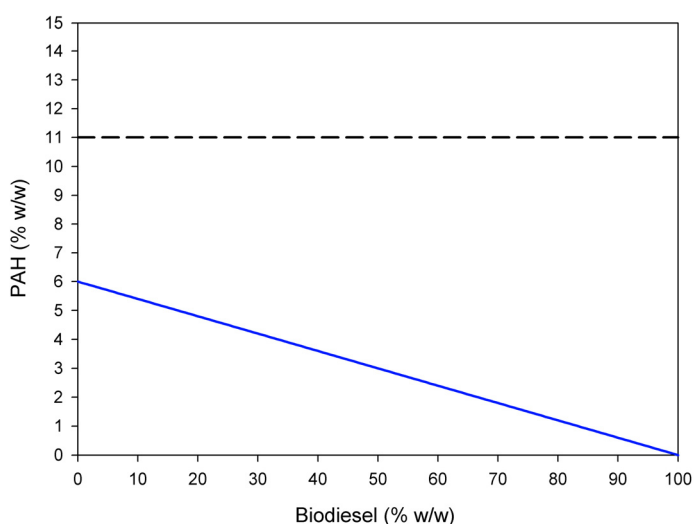Figure 3.6. Polycyclic aromatic hydrocarbons content of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Doted line represents the standard limit.

3.4.4.3.   LUBRICITY *(EN ISO 12156-1)*

Lubricity is the ability to reduce friction between solid surfaces in relative motion. The lubricity of a diesel fuel is measured using a high frequency reciprocating rig (HFRR) apparatus, where a steel ball applies a force to a disk submerged into the sample liquid at a determined temperature. The ball oscillates with a determined frequency and length, and creates a wear scar over the surface of the disk. The diameter of the wear scar (in μm) is measured under a microscope and reported as the lubricity parameter, being higher for less viscous liquids.

Biodiesel has good lubricating properties with wear scar diameters around 200 μm. However, biodiesel standards do not contain lubricity specifications. Traditional diesel fuel usually had good lubricating properties, but the treatments applied to reduce sulphur content in diesel fuel cause the removal of its most lubricating components. Thus, low and ultra-low sulphur diesel fuel need the addition of lubricity enhancers in order to satisfy the specifications.

Several studies show that the addition of low percentages of biodiesel (1-5%) to a low lubricity diesel fuel is enough to increase the lubricity to values that satisfy the diesel normative *(Knothe 2005, Goodrum 2005, Geller 2004).*

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3.4.4.4.   DISTILLATION *(EN ISO 3405)*

The distillation curve of a fuel indicates the percentage of that fuel that boils at a determined temperature. Fuels usually present three distillation ranges: low boiling compounds, which determine engine standard affect the flash point; medium boiling point compounds that characterise engine reaction; and high boiling compounds, or heavy compounds, that determine the power of the fuel.

The presence of extremely high boiling compounds is a cause of engine deposits, while low boiling compounds affect the flash point. Thus, the characteristics of the distillation curve of diesel fuel are important and the increase in the distillation temperature can give information on diesel fuel composition, storage behaviour and engine performance.

On the other hand, as most of the methyl esters usually contained in biodiesel (C16 to C18) have similar boiling points, the distillation curve for biodiesel presents almost constant temperatures *(Mittelbach 2004)*.

3.5. MIXTURES CHARACTERISATION

3.5.1. MATERIALS

Diesel fuel and biodiesel were provided by Petromiralles SL and Stocks del Vallés – BDP, respectively. Tables 3.8 and 3.9 present the quality parameters for both fuels. Mixtures with 5, 10, 15, 20, 25, 30, 40, 50 and 75 % (v/v) biodiesel were prepared.

Table 3.8. Quality parameters for diesel fuel from Petromiralles SL.

Parameter	Results	Method
Cetane number	51.2	EN ISO 5165
Cetane index	49.8	EN ISO 4264
Density at 15ºC	839.4 kg/m^3	EN ISO 3675
Density at 15ºC (in air)	838.3 kg/m^3	(calculated)
Polycyclic aromatic hydrocarbons	6 % (w/w)	EN 12916
Sulphur content	13 mg/kg	EN ISO 20884
Flash point	65 ºC	EN ISO 2719
Carbon residue	0.03 % (w/w)	EN ISO 10370
Ash content	0.003 % (w/w)	EN ISO 6245
Water content	127 mg/kg	ISO 12937
Total contamination	8 mg/kg	EN 12662
Copper strip corrosion	Class 1	EN ISO 2160
Oxidation stability	10 g/m^3	EN ISO 12205
Lubricity	440 μm	ISO 12156-1
Viscosity at 40ºC	2.66 mm^2/s	EN ISO 3104
Distillation	0 % (v/v) at 250ºC 93 % (v/v) at 350ºC 95 % (v/v) at 360ºC	EN ISO3405
Appearance	Clear and bright	ASTM D 4176
Colour	1	ISO 2049
CFPP	-13 ºC	EN 116
Cloud point	0 ºC	EN 23015
FAME content	0 % (v/v)	EN 14078

Table 3.9. Quality parameters for biodiesel from Stocks del Vallés - BDP.

Parameter	Results	Method
Water content	100 mg/kg	ISO 12937
Total contamination	15.08 mg/kg	EN 12662
Acid value	0.49 mgKOH/g	EN 14104
Monoglycerides	0.229 %(w/w)	EN 14105
Diglycerides	0.153 %(w/w)	EN 14105
Triglycerides	0.026 %(w/w)	EN 14105
Free glycerol	0.002 %(w/w)	EN 14105
Total glycerol	0.111 %(w/w)	EN 14105
Ash content	< 0.02 % (w/w)	ISO 3987

3.5.2. DENSITY

The density of diesel fuel, biodiesel and their mixtures was measured at 15ºC, using calibrated areometers, according to standard test method EN ISO 3675.

3.5.3. VISCOSITY

The viscosity of diesel fuel, biodiesel and their mixtures was measured at 40ºC, at least in triplicate. A Cannon-Fenske viscometer for transparent liquids, previously calibrated with distilled water was used, according to standard test method EN ISO 3104.

3.5.4. COLD FILTER PLUGGING POINT (CFPP)

The CFPP of diesel fuel, biodiesel and their mixtures was measured at least in triplicate and according to standard test method EN 116. Mixtures analysed contained 5, 10, 15, 20, 25 and 30% (v/v) biodiesel.

3.5.5. FLASH POINT

The flash point of diesel fuel, biodiesel and their mixtures was measured using a Pensky-Martens closed cup equipment. All the flash points were measured at least in triplicate and

according to standard test method EN 2719. Mixtures analysed contained 5, 10, 15, 20, 25 and 30% (v/v) biodiesel.

3.5.6. OXIDATIVE STABILITY

The oxidative stability of diesel fuel, biodiesel and their mixtures was measured at least in triplicate and according to standard test method EN ISO 12205. Mixtures analysed contained 5, 10, 15, 20, 25 and 30% (v/v) biodiesel.

3.5.7. CETANE INDEX

The cetane index of diesel fuel, biodiesel and their mixtures was calculated from density and distillation results, according to EN ISO 4264.

3.5.8. DISTILLATION

The distillation curve of diesel fuel, biodiesel and their mixtures was measured at least in triplicate and according to standard test method EN 3405. Mixtures analysed contained 5, 10, 15, 20, 25, 30, 40, 50 and 75% (v/v) biodiesel.

3.6. RESULTS AND DISCUSSION

3.6.1. DENSITY

The density of the mixtures of diesel fuel and biodiesel can be correlated by a lineal combination, where δ_i is the density of the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the volume fraction of diesel fuel or biodiesel in the mixture.

$$\delta_M = \delta_{DF} \cdot X_{DF} + \delta_{BD} \cdot X_{BD} \tag{3.12}$$

Equation 3.12 is helpful for the calculation of the maximum biodiesel percentage allowed in a mixture in order to satisfy diesel fuel normative. In the extreme case that the densities of both fuels are closed to the respective inferior limits (820 kg/m³ for diesel fuel and 860 kg/m³ for biodiesel), the maximum biodiesel percentage allowed should be 62.5% (v/v).

Figure 3.7. Density (at 15ºC) of biodiesel and diesel fuel mixtures. Symbols represent experimental results. Doted lines represent the standard limits.

3.6.2. VISCOSITY

The viscosity of the mixtures of diesel fuel and biodiesel can be correlated by different models for binary mixtures (see 3.4.2.4). The best results where obtained for the Grunberg and Nissan model (equations 3.7 and 3.8). However, this model is not a general correlation as it needs the determination of a factor for every different mixture. As the objective of this

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work was to obtain the most general model that best fits with experimental, the viscosity of the mixtures was adjusted using equation 3.5 for a binary mixture, being:

$$\mu_M = \exp(X_{DF} \cdot \ln \mu_{DF} + X_{BD} \cdot \ln \mu_{BD}) \tag{3.13}$$

Where μ_i is the viscosity of the mixture, diesel fuel or biodiesel (i: M, DF or BD respectively) and X_i is the volume fraction of diesel fuel or biodiesel in the mixture.
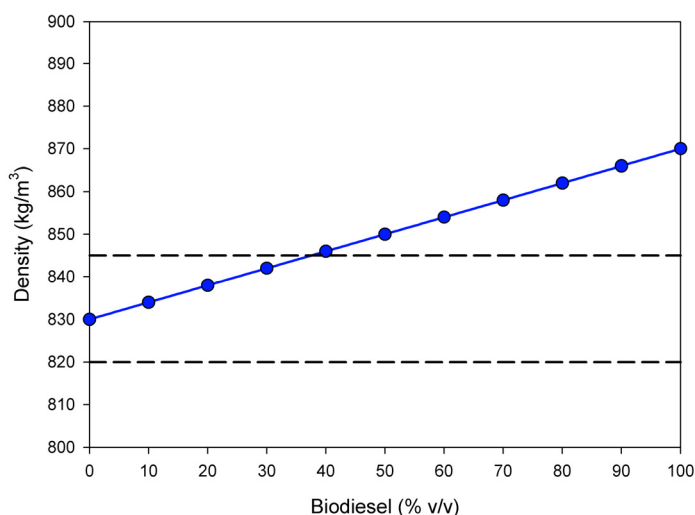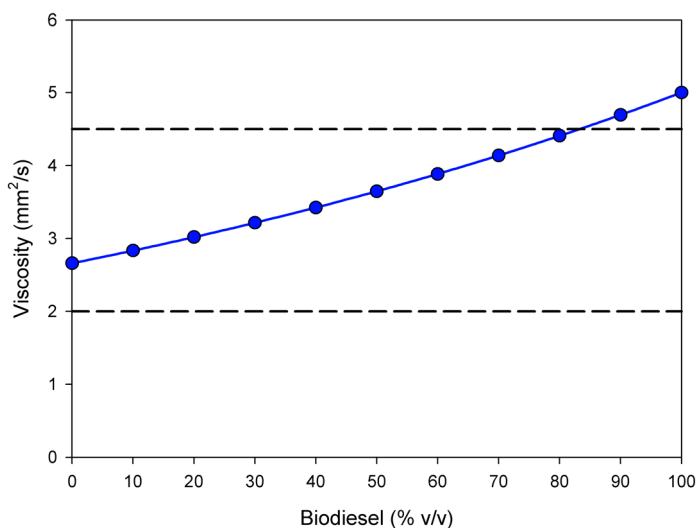

Figure 3.8. Viscosity (at 40ºC) of biodiesel and diesel fuel mixtures. Symbols represent experimental results. Doted lines represent the standard limits.

Equation 3.13 is helpful for the calculation of the maximum biodiesel percentage allowed in a mixture in order to satisfy diesel fuel normative. In the extreme case that the viscosities of both diesel fuel and biodiesel are closed to the respective inferior limits (2.0 mm^2/s for diesel fuel and 3.5 mm^2/s for biodiesel), all the mixtures will satisfy the specifications for diesel fuel. For higher viscosity values in diesel fuel, biodiesel or both of them, there will be a maximum biodiesel percentage allowed.

3.6.3. COLD FILTER PLUGGING POINT (CFPP)

Figure 3.9 shows the CFPP measured for diesel fuel, biodiesel and their mixtures. The CFPP value for the mixtures is included between the CFPP values for the original compounds. However more experiments are needed in order to determine an appropriate correlation.

Figure 3.9. CFPP of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Symbols represent experimental results. Doted lines represent the standard limits.

3.6.4. FLASH POINT

The flash point of biodiesel, diesel fuel and their mixtures is shown in figure 3.10. As the volatile components that determine the flash point of diesel fuel, are still present in mixtures with low biodiesel percentages, the flash point of the mixtures is closer to the flash point of the original diesel fuel.
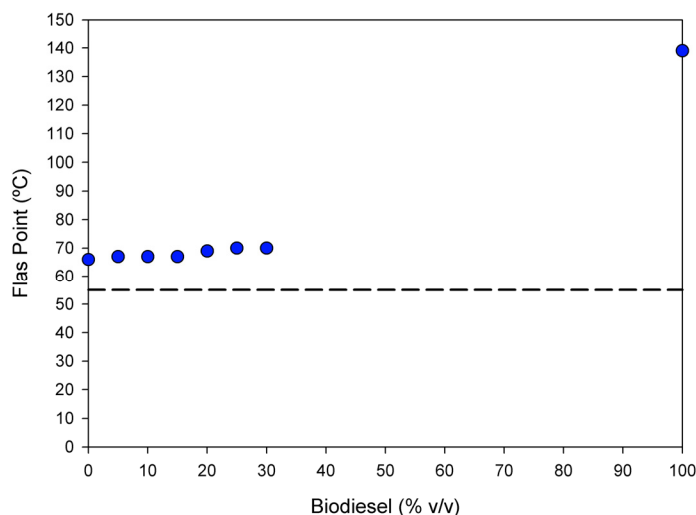
Figure 3.10. Flash point of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Symbols represent experimental results. Doted lines represent the standard limits.

3.6.5. OXIDATIVE STABILITY

Figure 3.11 presents the results for the oxidative stability of biodiesel-diesel mixtures, according to standard test method for diesel fuel. It seems that this method is not appropriate for biodiesel and its mixtures as there is no clear tendency. Biodiesel oxidation products may probably be soluble or pass through the filter used, thus giving wrong results.
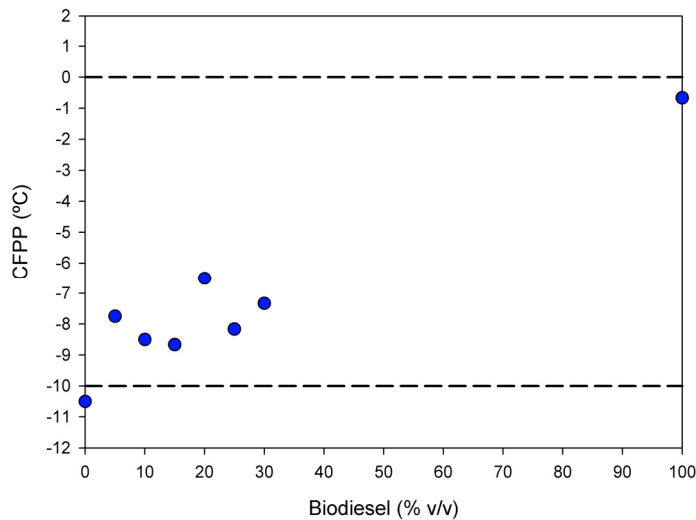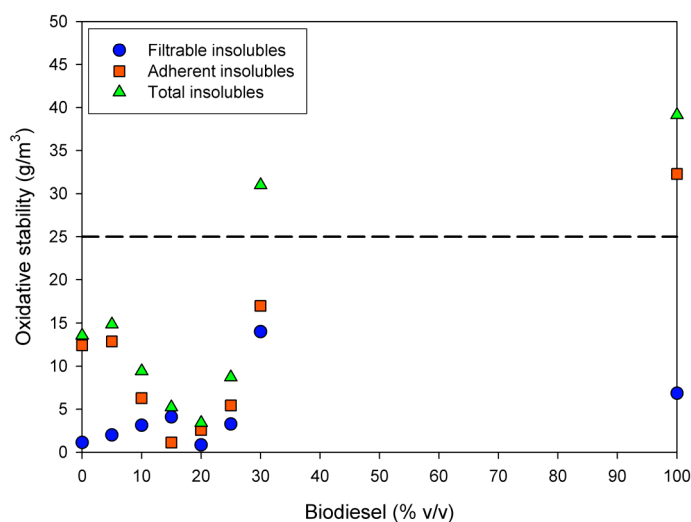

Figure 3.11. Oxidative stability of biodiesel and diesel fuel mixtures as a function of the biodiesel content. Symbols represent experimental results. Doted line represents the standard limit.

3.6.6. DISTILLATION

The distillation curves of pure diesel fuel and biodiesel are presented in figure 3.12. While diesel fuel components are distilled from 230 to 370ºC, the temperature range for biodiesel is sharp (330 to 380ºC) as there are few differences between the boiling points of the fatty acid methyl esters. The distillation curve of biodiesel does not give much information as almost all the components distille at the same temperatures.

Distillation curves of the biodiesel-diesel fuel mixtures are among the distillation curves of the pure components. Figures 3.13 and 3.14 present the variation of some distillation points with the % of biodiesel present. If diesel fuel specifications have to be met, only mixtures with low biodiesel % will be allowed.

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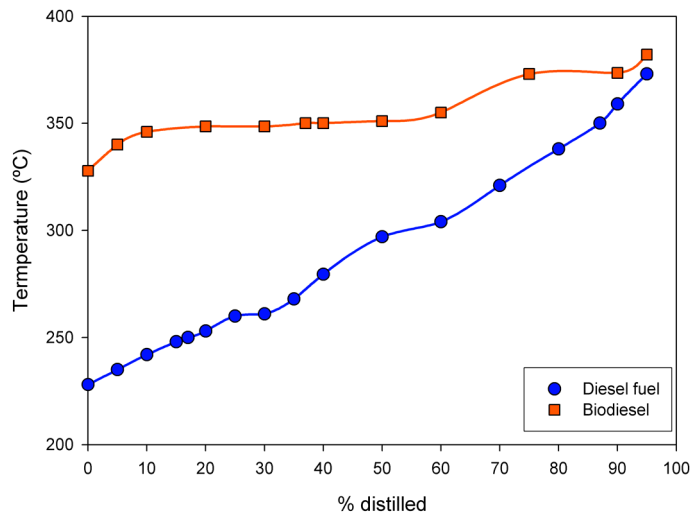
Figure 3.12. Distillation curves of biodiesel and diesel fuel.
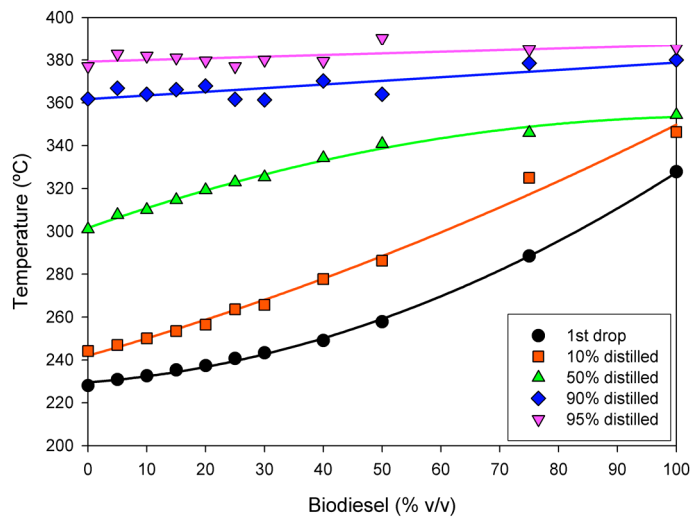
Figure 3.13. Some important distillation points for biodiesel and diesel fuel mixtures.
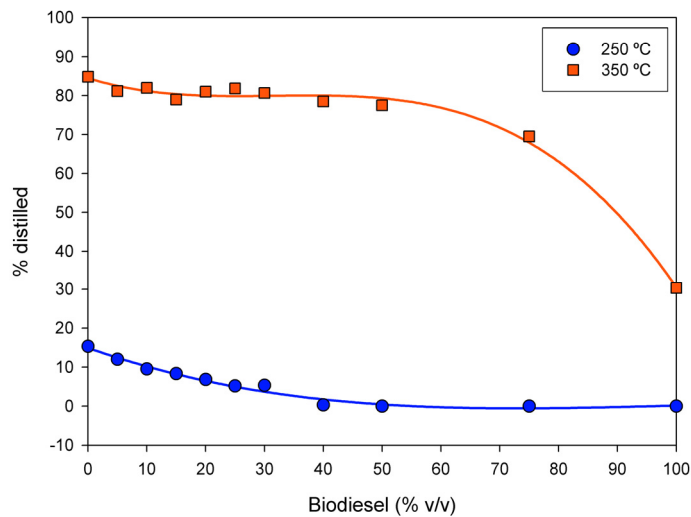
Figure 3.14. Percentages distilled at specific temperatures.

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3.6.7. CETANE INDEX

The cetane index was calculated for all the mixtures according to EN ISO 4264, using equation 3.6 and the values of the density and 50% temperature distillation obtained in 3.6.1 and 3.6.6.

Figure 3.15 shows the variation in cetane index with the % of biodiesel present in the mixture. Biodiesel usually presents higher cetane numbers than diesel fuel, however, when the cetane index is calculated, the tendency is inverse. As the correlation was developed for diesel fuel, it is not appropriate for its use with biodiesel.
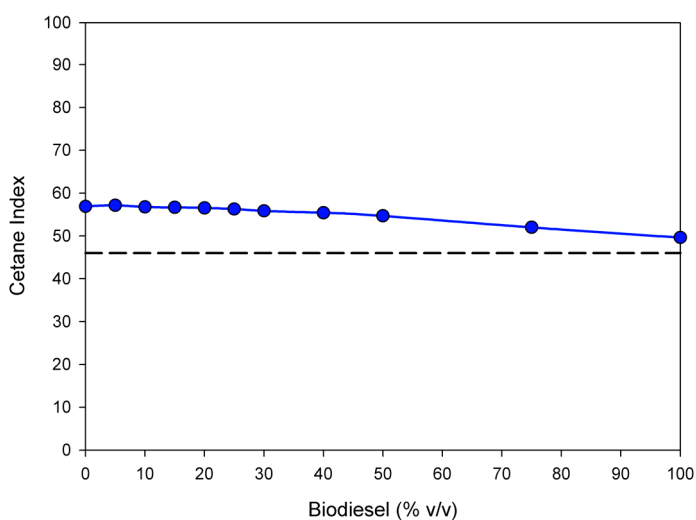
Figure 3.15. Cetane index of biodiesel and diesel fuel mixtures as a function of the biodiesel content, calculated according to diesel fuel correlation *(EN ISO 4264)* . Symbols represent experimental results. Doted line represents the standard limit.

3.7. CONCLUSIONS

There are some properties of the mixtures than can limit the amount of biodiesel allowed in the mixture in order to satisfy the specifications for diesel fuel:

➢ Density

➢ Ash content

➢ Water content

➢ Oxidative stability

➢ Viscosity

➢ Distillation

➢ CFPP

Among them, water and ash content should not represent a problem as biodiesel usually presents low values for these parameters.

The differences in the density and viscosity of diesel fuel and biodiesel, are caused by their differences in composition and should not be a limitation for the use of biodiesel. Appropriate specification limits are needed for the mixtures.

The distillation curve of biodiesel is not an important parameter for this fuel as it does not give relevant information. Thus, the distillation curve of the mixtures with diesel fuel should not be a limiting parameter for the use of biodiesel.

Appropriate test methods must be developed for the determination of oxidative stability of the mixtures, as the actual methods employed for the pure diesel fuel and biodiesel, are not applicable to their mixtures.

The CFPP is an important parameter for the use of biodiesel and its mixtures with diesel fuel, specially in cold weather conditions. This parameter is an important limitant of the amount of biodiesel present in a mixture.

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4. BIODEGRADATION OF BIODIESEL AND ITS MIXTURES WITH FOSSIL DERIVED FUELS

Bressol de vida,
camins de somnis,
pont de cultures
(ai, qui ho diria...!)
ha estat el mar.

Mireu-lo fet una claveguera.
Mireu-lo anar i venir sense parar.

Sembla mentida
que en el seu ventre
es fes la vida.
Ai, qui ho diria
sense rubor!

Mireu-lo fet una claveguera,
ferit de mort.

De la manera
que el desvalisen
i l'enverinen,
ai, qui ho diria,
que ens dóna el pa!

Mireu-lo fet una claveguera.
Mireu-lo anar i venir sense parar.

¿On són els savis
i els poderosos
que s'anomenen
(ai, qui ho diria!)
conservadors?

Mireu-lo fet una claveguera,
ferit de mort.

Quanta abundància,
quanta bellesa,
quanta energia
(ai, qui ho diria!)
feta malbé!

Per ignorància, per imprudència,
per inconsciència i per mala llet.

Jo que volia
que m'enterressin
entre la platja
(ai, qui ho diria!)
i el firmament!

I serem nosaltres (ai, qui ho diria!)
els qui t'enterrem.

Joan Manuel Serrat, Plany al Mar, 1984

4.1. INTRODUCTION

The risk of environmental contamination is present along the different steps of petroleum and its derivatives life cycle. Accidental spills occur during the extraction, transportation, refining, storage, usage and ultimate disposal of these fuels. They can cause serious damage to marine environments, soil and sediments, and affect the ecology, the economy and the society of the contaminated regions. Common accidents can involve motorised vehicles, leaks from underground storage tanks, pipelines, illegal disposals, etc *(Boopathy 2004, Molina 2004, Mukherji 2004, Marchal 2003).*

Fortunately, the incidence of large spills is relatively low and has decreased significantly during the last 30 years (figures 4.1 and 4.2). Small spills (less than 700 tonnes) are more frequent, but their contribution to the total quantity of petroleum products spilled to the environment is small *(ITOPF, 2006).*

Small spills are mainly caused by routine operations, such as loading, discharging and bunkering in ports or oil terminals. These operational spills usually involve less than 7 tonnes of petroleum products. Larger spills are mainly caused by accidents such as collisions, groundings, hull failures, fire and explosions *(ITOPF, 2006).*

The physical and chemical processes involved in the dissipation of the spill into the marine environment is called weathering and is described in figure 4.3.

When a petroleum product is spilt in the sea it spreads out over the surface. Its viscosity and the water conditions affect the spreading speed. The most volatile compounds of the product evaporate during the first days of the spill. The waves and turbulence of the sea help the formation of droplets that disperse in the water and favour the dissolution, biodegradation and sedimentation of the product. Turbulence also helps the formation of emulsions that increase the volume of contaminant and persist more than the original product. Water soluble compounds, such as light aromatic hydrocarbons, dissolve into the water. Oxygen and sunlight promote the oxidation of some components and the formation of tars, increasing the contaminant persistence. Some heavy components are dense enough to sink in the water and form sediments. The microorganisms present in the water can partially or completely degrade the product to CO_2 and water or water soluble compounds. However, some compounds are persistent or degrade very slowly. Biodegradation depends on the level of oxygen, nutrients and temperature. It occurs at the petroleum-water

interface, because there is no oxygen present within the petroleum, so natural and chemical dispersion increase the surface area and favour biodegradation *(ITOPF, 2002)*.
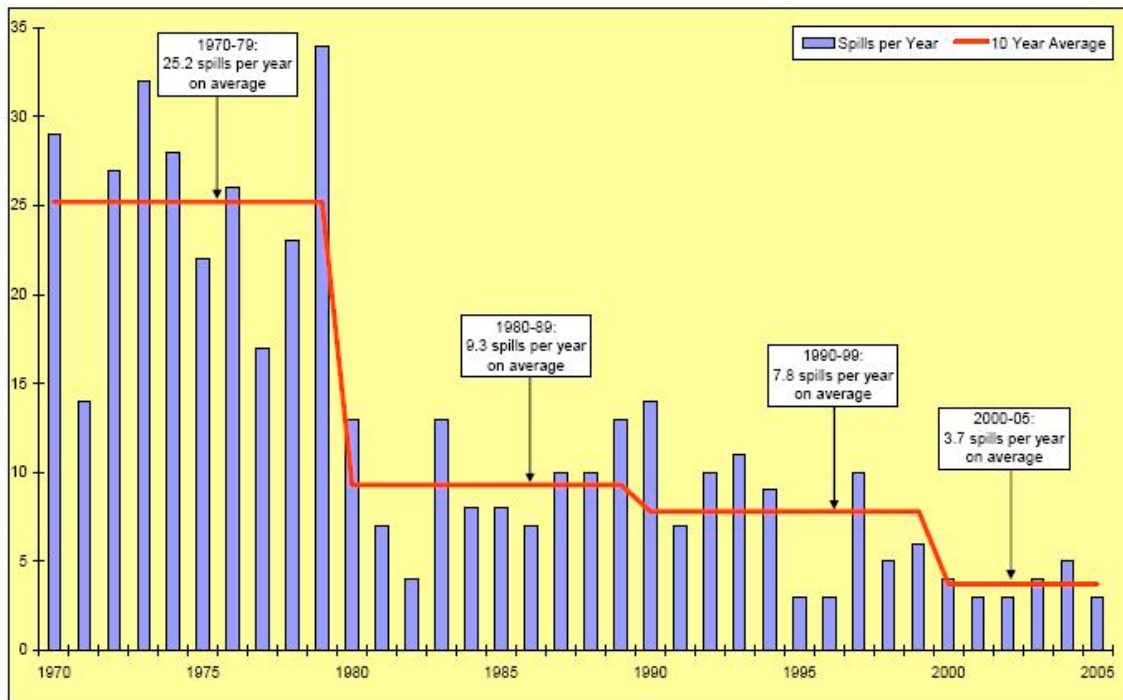

Figure 4.1. Number of spills over 700 tonnes *(ITOPF 2006)*
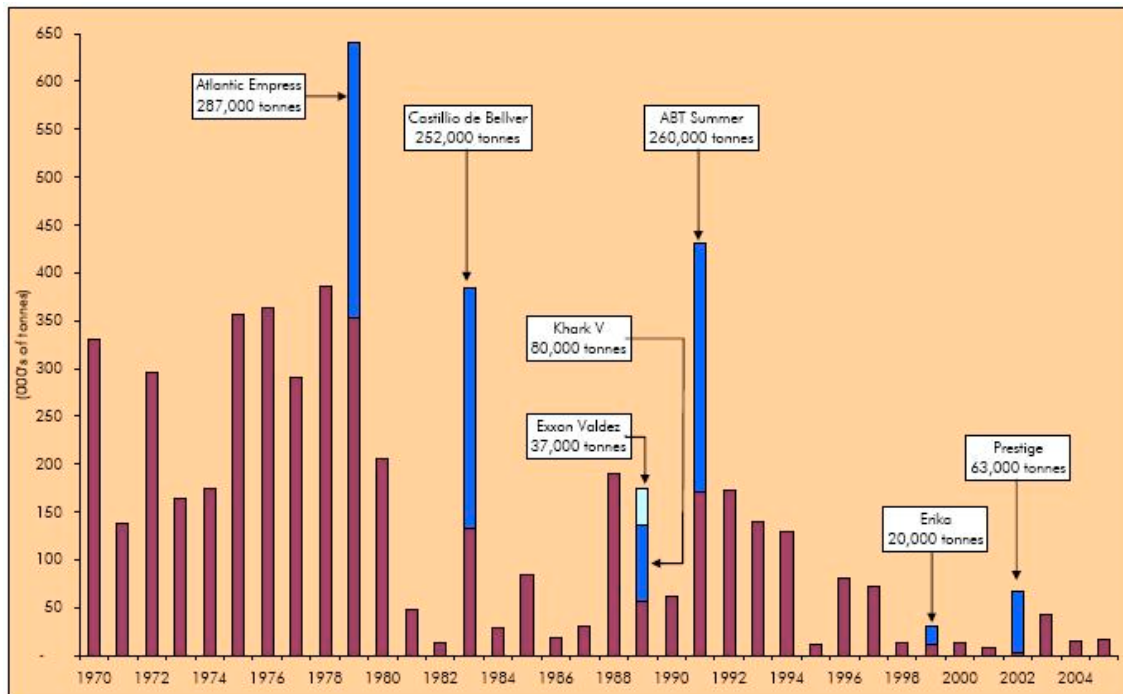
Figure 4.2. Quantities of oil spilt *(ITOPF 2006)*

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Weathering is affected by the physical properties of the product spills, such as density, viscosity and pour point. Light products such as kerosene, evaporate and dissipate quickly but persistent products require a clean-up response *(ITOPF, 2002)*.
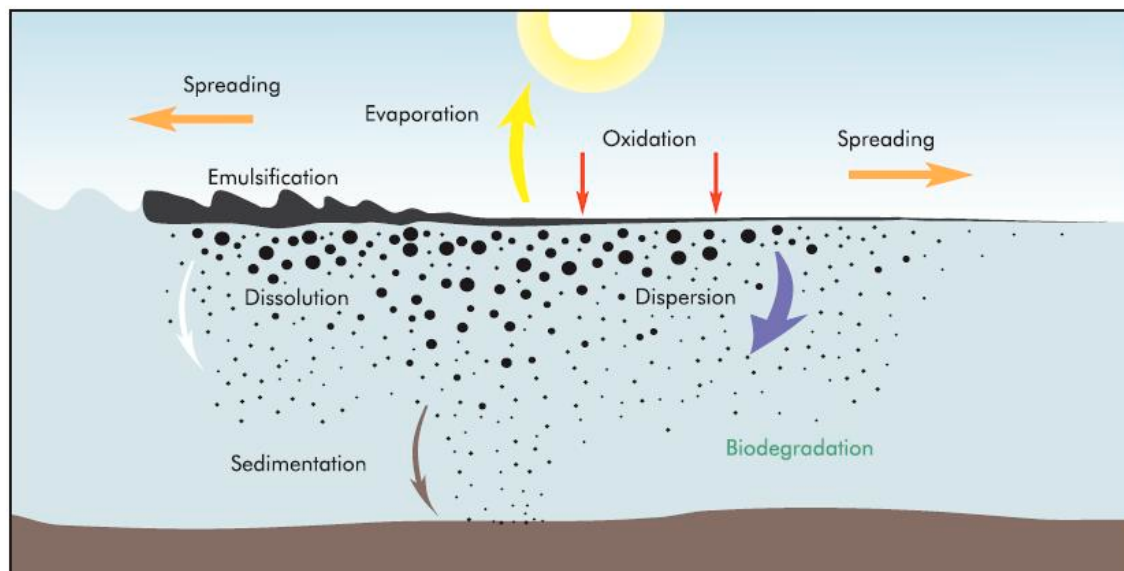
Figure 4.3. Processes acting on spilled oil *(ITOPF 2002)*

4.1.1. CRUDE OIL SPILLS

Some examples of accidental crude oil spills in the sea are the Amoco Cadiz that spilt 230000 tonnes of crude oil in the Britain and French costs (1978), the Exxon Valdez that spilt 37000 tonnes of crude oil in Alaska (March 1989), the Erika with 20000 tonnes of crude oil on the French coasts (December 1999), and recently the Prestige, which sank in the Atlantic Ocean of the North West coast of Spain (November 2002). With 63000 tonnes of heavy crude oil spilt, the Prestige has situated in the 20th place in the rank of mayor oil spills. It contaminated 1900 km of shorelines, mostly in Spain, affecting fisheries and tourism. More than two years, 100 million Euro and 5000 people were needed to clean and recover the area. Most of the work was done manually, collecting 16000 tonnes of oil waste *(CEDRE-a 2003, ITOPF 2006)*.

The product transported by the Prestige was a heavy crude oil, a residue from crude petroleum distillation, after the volatile fractions had been eliminated. It could be used in industrial combustion, like thermal power stations or furnaces, and the ships driven by slow diesel engines. It contained 86.8 % carbon, 11 % hydrogen, 2.28 % sulphur and 0.69

% nitrogen. It had a density of 990 kg/m^3 at 15 ºC, and a viscosity of 615 cp at 50 ºC and 30000 cp at 15 ºC *(CEDRE-b 2003)*. Because of all this and its low solubility and volatility, it persisted in the environment. In fact, samples collected at the beaches two weeks after the spillage showed no signs of evaporation or transformation. The viscosity is an important parameter when choosing the forms of fight in the sea and the cleaning of the coast. When the oil reaches the shore the removal becomes more difficult, more expensive and more time consuming *(Pereira 2004)*. After the oil spill occurs, only the lightest fraction vaporises while the heaviest one is slowly biodegraded *(CSIC-a 2003)*. This type of crude oil degrades very slowly in the sea, about 12 % in a month. This makes it necessary to collect most of the oil manually before other cleaning proceedings are applied *(CSIC-b 2003)*. After the physical removal, different technologies can be applied for the complete restoration of the area, like chemical or biological treatments and combinations of them *(Molina 2004)*. Chemical treatments usually involve the use of organic solvents with or without surfactants, but they can cause toxicity in the aquatic organisms *(Pereira 2004)*. An ecologically acceptable technology is bioremediation that uses microorganisms to degrade pollutants such as oil and oil products *(Molina 2004)*. In fact, it can be estimated that a spill would normally take five to ten years to return to normal conditions and this time can be reduced to a period of two to five years, through the use of bioremediation technology, as happened in the Exxon Valdez disaster. However, bioremediation is the ultimate fate of any oil not collected and is recommended for use following the physical removal. This focuses the interest in effective low cost technologies that accelerate the removal of the oil from beaches without causing equivalent or greater damage *(Pereira 2004)*.

4.1.2. CRUDE OIL DERIVATIVES

Several authors have reported the biodegradation of fossil diesel fuel alone. On site bioremediation of diesel fuel contaminated soil in the Arctic, gave 90-95 % degradation after 365 days *(Mohn 2001)*. In artificially contaminated soils, 67% degradation can be observed after 109 days *(Molina 2004)*. The biodegradation of diesel fuel was also determined in aquatic environments, using an isolated culture obtained from the vicinity of an oil field. Under aerobic conditions the maximum biodegradation was obtained after 30 days with only 42 % *(Mukherji 2004)*. Studies on soil columns reveal that more than 300 days are required to treat a diesel contaminated soil under anaerobic conditions *(Boopathy 2004)*. In aquatic environments, only 18% biodegradation is detected after 50 days, what

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confirms that anaerobic biodegradation is slower and less effective than aerobic *(Mukherji 2004)*. Mixtures of biodiesel and fossil diesel fuel show that biodiesel can promote and speed up the biodegradation of diesel by means of cometabolism *(Zhang 1998)*.

Another fossil-derived fuel that usually contaminates soil and aquatic systems is gasoline. The major gasoline constituents (benzene, toluene, ethylbenzene and xylene – BTEX) are relatively soluble in water and are considered human carcinogens *(Duarte-da Cunha 2000)*. Although BTEX compounds are particularly difficult to degrade because of the lack of an activating oxygen or nitrogen substituent group, they biodegrade completely in less than 150 days *(Phelps 1999)*.

4.1.3. BIODEGRADATION OF BIODIESEL

Several studies show the high biodegradability of biodiesel alone, in freshwater and soil environments. Its primary biodegradation is complete after 7 days under aerobic conditions, and after 14 days under anaerobic conditions. A mineralization of about 90-98 % occurs in 21-28 days under both aerobic and anaerobic conditions. Biodiesel samples from different origins have proven to be readily biodegradable according to EPA standards, and the biodegradation rate in the aquatic environment is high *(Makareviciene 2003, Zhang 1998)*.

4.1.4. BIOREMEDIATION TECHNOLOGY

Effectiveness of bioremediation depends on the ability of the microorganisms to degrade the substrate, the environmental scenario and other factors such as temperature, pH, availability of nutrients and the presence of electron acceptors. Microorganisms collected from historically contaminated habitats have enhanced oil degrading abilities *(Mukherji 2004)*. In situ bioremediation technologies involve the stimulation of naturally occurring microorganisms so that they can degrade the organic contaminants present in the environment *(Boopathy 2004)*.

As very few organisms degrade a simple compound completely, degradation of organic compounds is usually carried out by community-interacting microbial populations *(Boopathy 2004)*. Laboratory studies are typically conducted with individual organic substrates, but

natural and polluted environments characteristically contain a multiplicity of organic compounds that can be used by one or more of the indigenous bacteria or fungi. One substrate often enhances the degradation rate of the other. The reasons for this behaviour are largely unknown and have hardly been explored. Some hypotheses are that *(Alexander 1994)*:

a) The additional carbon source increases the population size,

b) A large mass of cometabolizing cells is produced,

c) Growth factors are excreted by the population acting on the second compound,

d) The enzymes necessary for the degradation of the first compound are produced.

Cometabolic transformation (cometabolism) is the process in which microorganisms use a second substrate, which is readily biodegradable, as the carbon or energy source to degrade the first substrate. This transformation is very important for the degradation of resistant substances or when the microorganisms present are not able to attack the substrate if it is the sole carbon source *(Zhang 1998)*. Then, cometabolic transformation can be used to the eliminate resistant substances, such as crude oil and its derivatives.

4.1.5. BIODIESEL APPLICATIONS

Some studies suggest that biodiesel can be used as a biological solvent and that it may remove twice the amount of oil from sand, compared to conventional shoreline cleaners. Biodiesel can be produced in large quantities at a low cost, and is also less toxic than crude oil or its derivatives, being rapidly degraded in the environment *(Pereira 2004)*. Biodiesel dissolves the crude oil, reducing its viscosity and facilitating the collection. The surface area of the oil is also increased, thus facilitating microbial degradation *(CSIC-a 2003)*.

In some studies biodiesel was used as a solvent to clean sand contaminated with crude oil in microcosms and mesocosms *(CSIC-a 2003, Mudge 1999, Pereira 2004)*. The mixtures were then washed with water and, in all the cases, most of the crude oil was removed from the sediments after the addition of relatively small volumes of biodiesel (1:2 or 1:1 biodiesel:crude oil relations, depending on the type of sediment). In another study *(Taylor 2001)*, biodiesel was used as a dispersant to enhance the bioremediation of coal tars. The

results suggest that biodiesel can solubilise and increase the bioavailability of coal tar polycyclic aromatic hydrocarbons in soils.

If biodiesel has to be spread in a particular area, the operation is low cost and does not require high technology. If this operation also proves to be environmentally friendly, it will have many possible applications in the treatment of affected areas. However, there is no much information about the biodegradability and toxicity of the biodiesel-crude oil mixture that is left in the environment, but it appears to have a low persistence as the biodiesel present in the mixture provides an available carbon source for the microorganisms that then may cometabolise the crude oil *(Pereira 2004)*.

In this work, the possibility of using biodiesel to cometabolically degrade a crude oil with similar properties to that of the Prestige oil spill has been studied. The changes in the density and viscosity of the crude oil after biodiesel is added are important for evaluating the best method of collecting the mixture. In addition, if biodiesel increases the biodegradability of the crude oil, any remains of the mixture left in the affected area will be degraded in a short time. The biodegradation of mixtures of biodiesel with commercial diesel fuel and gasoline has also been evaluated. This is important, not only for the use of biodiesel in the remediation of an area contaminated with diesel, but also in case of an accidental spill of the mixture of biodiesel and diesel that is commercially available at fuel stations in several countries, particularly Spain.

4.2. BIODEGRADATION METHODOLOGY

Knowledge of the biodegradability of chemicals is one of the most important aspects of their environmental behaviour because a biodegradable substance is expected to cause less ecological problems in the long term than a persistent one.

Biodegradation can be defined as the biologically catalysed reduction in complexity of chemicals. In the case of organic compounds, biodegradation frequently leads to the conversion of much of the C, N, P, S and other elements in the original compound to inorganic products. Such a conversion is known as "mineralization" or "ultimate biodegradation". To determine mineralization, the parameters that are frequently measured are the dissolved organic carbon (DOC), the biochemical oxygen demand (BOD) or the formation of CO_2. The advantages to measure these parameters are their relatively simple method of determination and the few techniques and apparatus required to cover an immense variety of organic substances. Their disadvantage is, however, that they do not take into account the transformation of the test material into biomass. The test result is obtained by comparing the measured parameter with a theoretical value, calculated form the molecular formula of the test compound. To measure the primary biodegradation, the only techniques that can be used are chromatography (GC or HPLC).

Biodegradation test results can be influenced by different models of a variety of environmental situations. This makes difficult to define a *true* method, and different results can be obtained using different methods or conditions. However, a test material that is normally easily or poorly biodegradable, will produce similar test results in all the cases. When comparing different test material under the same method and conditions, the results can give several valid conclusions, although the method used could not be the best for testing a single material *(Pagga 1997)*.

The CO_2 evolution test, which is based on mineralization is relatively simple, economic (equipment and reagents), environmentally safe an easy to develop, although time consuming. Under aerobic conditions and nutrient supply, microorganisms will metabolise a substance to CO_2 and water. This method considers CO_2 to be the prevalent indicator of organic substance breakdown. If the substrate is the only carbon source, the CO_2 produced will be proportional to the carbons consumed by microorganisms from the test substrate.

Then, the percentage of CO_2 evolution is proportional to the percentage of substrate degradation *(Zhang 1998).*

The method is normally conducted manually, and although automated tests have also been developed being more reproducible and less time consuming *(Calmon 2000),* the manual methods have more applications because of their uncostly apparatus *(Szaraz 2003).*

One important factor, which cannot be standardised, is the inoculum, the source of the microorganisms for the test, and their state of acclimatisation and adaptation. In standard tests, which are often used for a general prediction, activated sludge from municipal wastewater treatment plants is normally used *(Pagga 1997).*

In this work, it is tested the biodegradability of several biodiesel-crude oil, biodiesel-diesel and biodiesel-gasoline mixtures, using the CO_2 evolution test *(EPA 712-C-96-075).* The three fossil fuels are miscible with biodiesel in all proportions.

The biodegradability of biodiesels produced from different types of oils, such as sunflower, corn, Cynara cardunculus and recycled frying oil is also analysed.

Test flasks were prepared, consisting of 500-ml erlenmeyers with 225 ml of mineral nutrient medium, 25 ml of inoculum and an amount of the test substrate that contained an equivalent of 10-20 g carbon. All substrates were analysed in triplicate.
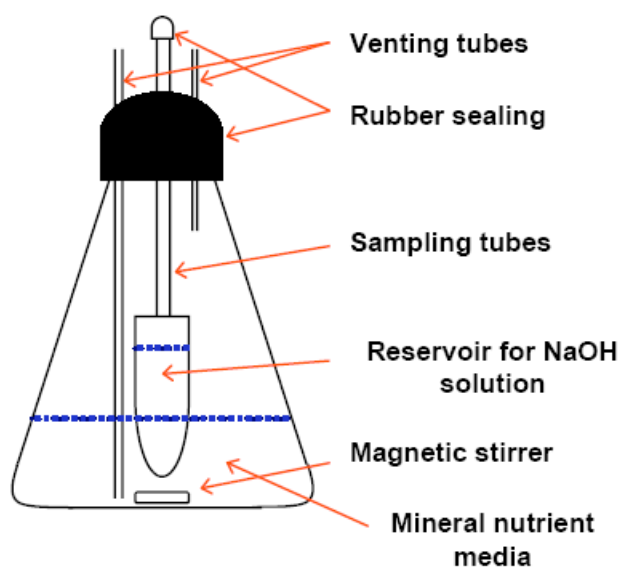

Figure 4.4. CO_2 evolution test. Experimental setup scheme

Three control flasks were prepared to measure the amount of detected CO_2 that did not correspond to the substrate. These flasks consisted of 500-ml erlenmeyers containing 225 ml of mineral nutrient medium and 25 ml of inoculum.

The mineral nutrient medium contained 0.5 g K_2HPO_4, 1.0 g NH_4Cl, 2.0 g Na_2SO_4, 2.0 g KNO_3, 0.2 g $MgSO_4.7H_2O$ and traces of $FeSO_4.7H_2O$ per litre of distilled and deionised water (DIW).

Because of limitations in space and material, the biodegradability of the samples was analysed in separate experiments, with time lags between them (table 4.1). The procedure employed was the same for all the experiments, though the inoculum could have different conditions.

Table 4.1. Biodegradation experiments performed using the CO_2 evolution test.

Experiment	Substrates analysed	Date
1	Biodiesel from sunflower, corn, Cynara cardunculus and residual cooking oils.	19/March/03 – 16/April/03 16/June/03 – 14/July/03
2	Biodiesel – petroleum mixtures with 0, 25, 50, 75 and 100% (w/w) biodiesel	19/March/03 – 16/April/03
3	Biodiesel – petroleum mixtures with 0, 12.5, 37.5, 62.5, 87.5 and 100% (w/w) biodiesel	16/June/03 – 14/July/03
4	Biodiesel – diesel mixtures with 0, 5, 12.5, 20, 25, 37.5, 50, 62.5, 75, 87.5 and 100% (w/w) biodiesel	02/August/04 – 30/August/04
5	Biodiesel – gasoline mixtures with 0, 17, 33, 50, 67, 83 and 100% (w/w) biodiesel	26/October/04 - 23/November/04

Sunflower, corn and Cynara cardunculus biodiesels were prepared in the laboratory with methanol (with a 6:1 methanol to oil molar relation) and using 1% NaOH as catalyst (with respect to the weight of oil). Biodiesel from used oil was provided by BIONET Europa S.L. This type of biodiesel was used to prepare the mixtures with crude oil, diesel and gasoline. The crude oil used, was a type with similar properties to that spilt by the Prestige. The diesel fuel and gasoline were obtained from local fuel stations (Repsol and AGIP, respectively). Elementary analysis of the samples is shown in table 4.2.

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Table 4.2. Elementary analysis of the substrates analysed

Element	Biodiesel	Crude Oil	Diesel fuel	Gasoline
C	76.9 ± 0.1^{b}	86.3 ± 0.3	83.4 ± 0.6	87.3 ± 0.2
H	13.7 ± 0.1	11.7 ± 0.3	14.6 ± 0.3	12.7 ± 1.5
N	0.0	0.5	0.0	0.0
S	0.0	1.9	0.0	0.0
O[a]	9.4	0.0	2.0	0.0

[a] Calculated by difference

[b] Confidence interval $\alpha = 0.01$

The inoculum consisted of activated sludge provided by the municipal water treatment plant in Tarragona, Spain, and was collected before the beginning of each set of experiments. This sludge was not specially prepared for these experiments, as we wanted to check how the mixtures behaved in a general aquatic environment and that they can be treated with any activated sludge present at a water treatment plant. As indicated in the method, it was previously acclimated with mineral nutrient medium and a small amount of the test substrate, and kept under permanent agitation for two weeks. Degradation was monitored over 28 days by determining the CO_2 that had been captured by a suspended reservoir containing a NaOH 0.1 M solution. A syringe was used to remove the solutions from the reservoirs at predetermined periods for titration. The reservoirs were then washed and refilled with fresh NaOH solution. The NaOH removed was combined with the rinse water and analysed by back-titration with HCl (0.1 N), after $BaCl_2$ had been added to precipitate the carbonate. Phenolphthalein was used as indicator.

The percentage of biodegradation was calculated by comparing the carbon in the trapped CO_2 to the amount of carbon in the initial substrate, corrected by the control flask results.

$$\%CO_2 = \frac{100(CF - TF)}{Ct} \qquad (4.1)$$

TF: volume of HCl (ml) required to titrate NaOH samples from the test flasks

CF: volume of HCl (ml) required to titrate NaOH samples from the control flasks

Ct: theoretical amount of HCl required to titrate the total carbon present in the substrate, in millilitres.

$$Ct = \frac{2m_S C_\%}{(C_{HCl} M_C)}$$

(4.2)

m_S: mass of substrate present in the flask (mg)

$C_\%$: percentage of carbon present in the substrate, determined by elementary analysis

C_{HCl}: concentration of the HCl solution (mol/ml)

M_C: molecular weight of carbon (mg/mol)

2: stoichiometric factor, as a mol of CO_2 will consume 2 moles of HCl

The total % CO_2 is the sum of the percentage obtained in each analysis and the previously accumulated amount.

$$Ct = \frac{2m_S C_\%}{(C_{HCl} M_C)}$$

4.3. PHYSICAL PROPERTIES OF THE MIXTURES

The viscosity of the pure biodiesels and the mixtures with diesel fuel and gasoline was measured using a Cannon-Fenske viscometer for transparent liquids, previously calibrated with distilled water. All the viscosities were measured at least in triplicate and at three different temperatures: 4 °C (cold sea water temperature), 15°C (normative specification for biodiesel and diesel fuel densities, EN ISO 3675) and 40°C (normative specification for biodiesel and diesel fuel viscosities, EN ISO 3104).

The viscosity of the mixtures of biodiesel and crude oil was measured using a rotational viscometer (Viscotester VT550), with the sensor system MV1, which is suitable for its use with medium viscosity liquids, working in the medium shear rate range. The VT550 has ten speed programs, each of which has six different speed levels. The program selected depends on the liquid's viscosity and the speeds vary from 0.5 min-1 for the lower programs, to 800 min-1 for the higher ones. The viscosity of all the samples was measured at the six speed levels of the appropriate program and at three different temperatures.

The density of the pure biodiesels and the mixtures with diesel fuel and gasoline was measured using calibrated areometers. The density of the mixtures of biodiesel and crude oil was measured using a 50-ml graduated test tube, previously calibrated with distilled water at the desired temperatures. To calculate density, mass and volume were measured for all the samples by triplicate and at three different temperatures: 4 °C (cold sea water temperature), 15°C (normative specification for biodiesel and diesel fuel densities, EN ISO 3675) and 40°C (normative specification for biodiesel and diesel fuel viscosities, EN ISO 3104).

4.4. RESULTS AND DISCUSSION

4.4.1. BIODEGRADATION BEHAVIOUR

Figures 4.5 to 4.9 show the results for the different experiments. There was a time lag between the sets of experiments, using different starting sludges (different environmental conditions affect the characteristics of the sludge). It is better to obtain previously activated sludge to improve the biodegradation kinetics. However, we wanted to check that using a locally available, non specific sludge, the biodegradation could occur with acceptable results. Indeed, there are differences between the kinetics of the separate experiments (due to the adaptation time of the sludge) but the final biodegradation behaviour is quite similar.
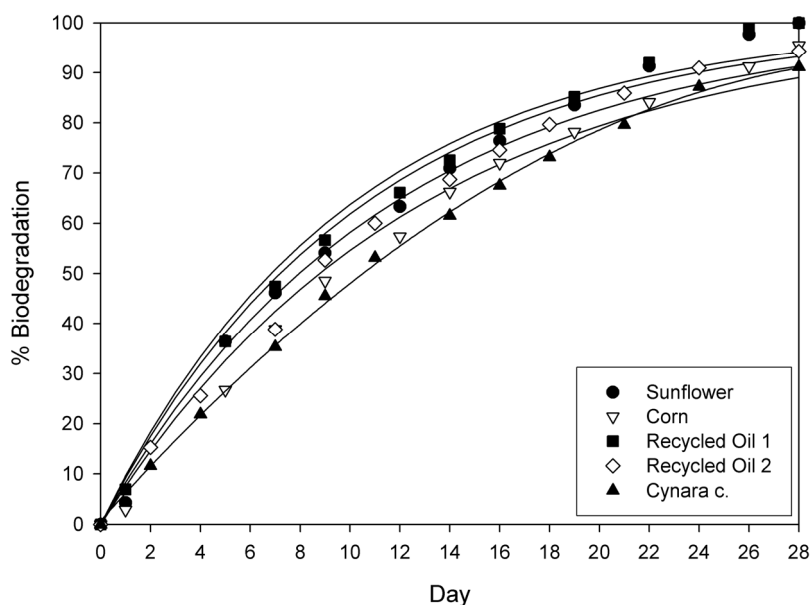
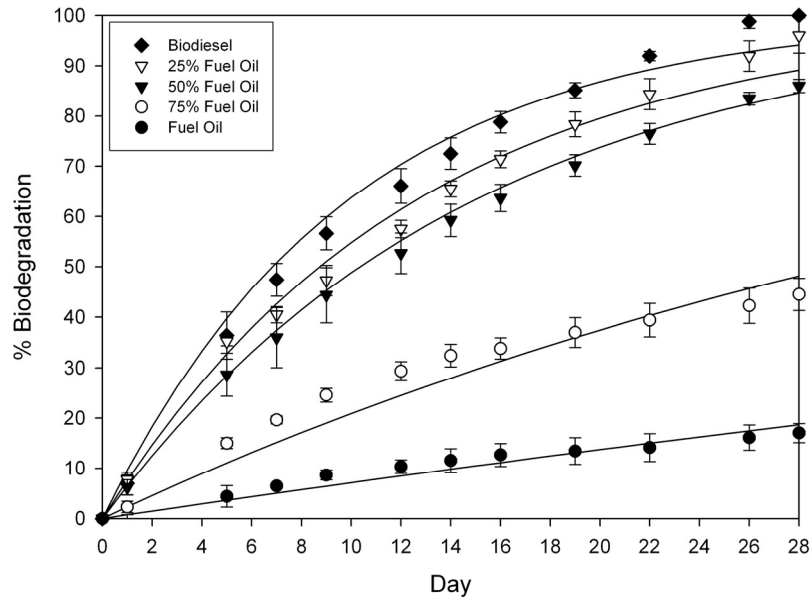
Figure 4.5. Biodegradation of different types of biodiesel

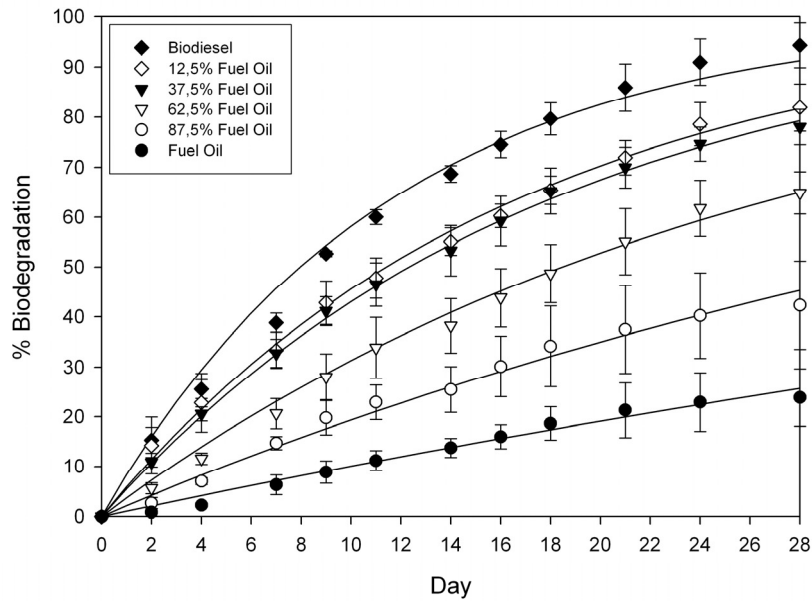Figure 4.6. Biodegradation of biodiesel-crude oil mixtures (experiment 2)

Figure 4.7. Biodegradation of biodiesel-crude oil mixtures (experiment 3)
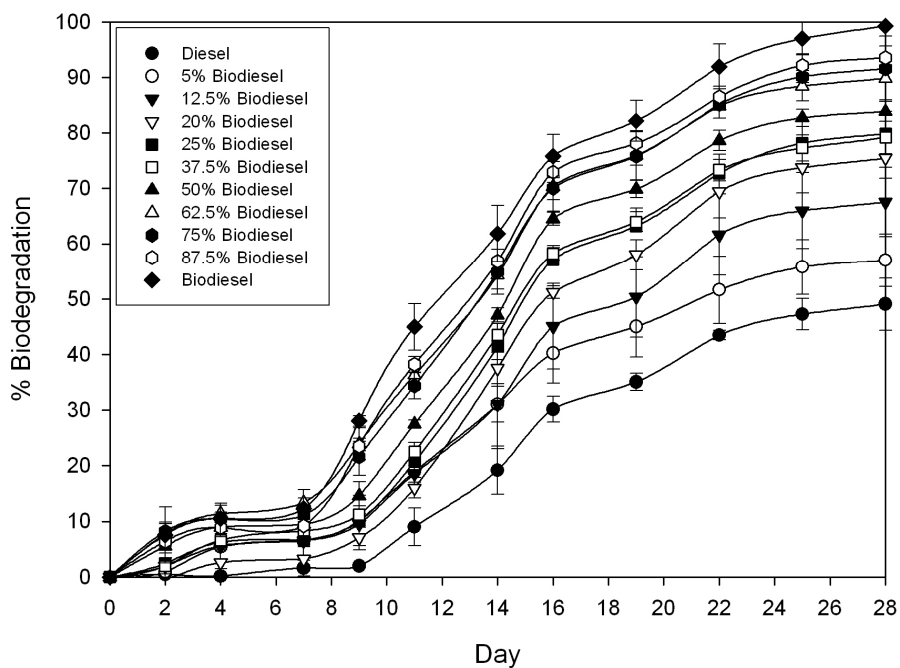
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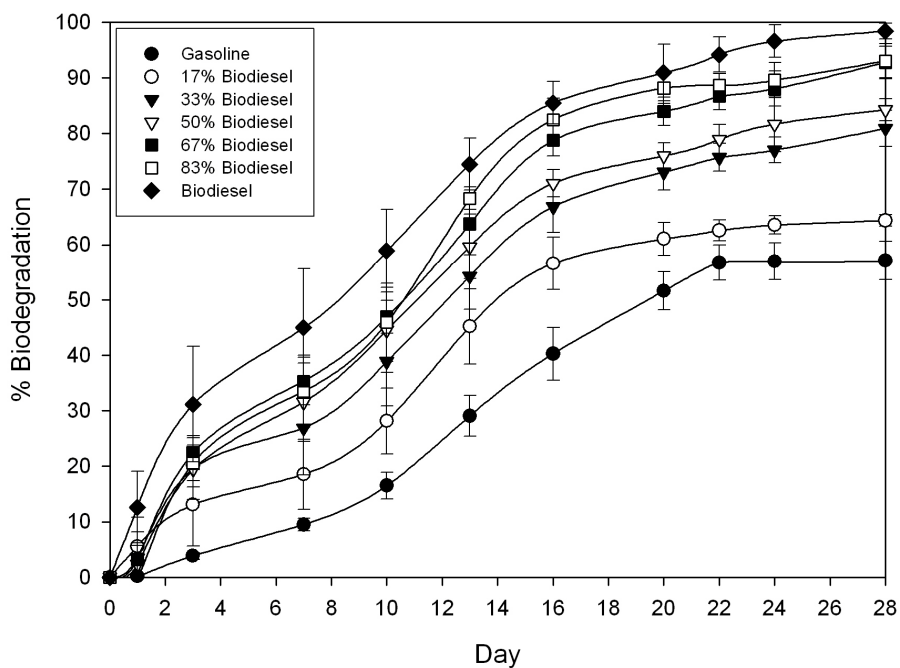Figure 4.8. Biodegradation of biodiesel-diesel mixtures

Figure 4.9. Biodegradation of biodiesel-gasoline mixtures

The biodegradation of the different biodiesels after the 28-day period presents similar behaviour. In all the cases, more than 90% of the biodiesel was degraded. In the experiments with mixtures the biodegradability of pure biodiesel reached nearly 100 % during the different test periods, while crude oil, diesel fuel and gasoline reached 20, 50 and 56% respectively. The biodegradability of the mixtures increased in all the cases when biodiesel was added.

A synergic effect was evaluated for all the mixtures in order to determine how adding biodiesel could improve the biodegradability of both fossil derived fuels. A linear combination was calculated, as follows:

$$LC = P_{FF} B_{FF} + P_{BD} B_{BD} \tag{4.3}$$

where:

LC: linear combination of biodegradability

P_{FF}: % of fossil derived fuel (crude oil, diesel or gasoline) in the mixture

P_{BD}: % of biodiesel in the mixture

B_{FF}: biodegradability of the pure fossil derived fuel (crude oil, diesel or gasoline)

B_{BD}: biodegradability of the pure biodiesel

This linear combination was compared with the experimental biodegradability value of the mixture, during the days of the experiment. The synergic effect is calculated from the comparison of these curves as follows:

$$S_{\%} = \frac{B_{exp} - LC}{B_{BD} - LC} \cdot 100 \tag{4.4}$$

where:

$S_{\%}$: synergic effect

B_{exp} : biodegradability of the mixture, found experimentally

B_{BD} : biodegradability of the pure biodiesel

LC : linear combination of biodegradability

If the experimental curve coincides with the linear combination curve, the synergic effect is cero, indicating that biodiesel has no effect on the biodegradability of the fuel.

If the experimental curve is above the linear combination curve, the synergic effect is positive, indicating that biodiesel improves the biodegradability of the fuel. The closer the experimental curve is to the pure biodiesel curve, the higher the synergic effect is.

If the experimental curve is under the linear combination curve, the synergic effect is negative, indicating that biodiesel worsens the biodegradability of the fuel.

Table 4.3 shows the synergic effect calculated for all the mixtures. For most of them, the synergic effect was positive, showing that biodiesel improves the biodegradation of both, crude oil and diesel, by means of cometabolism. The mixtures with higher biodiesel percentages present the lowest, or even negative, synergic factors. This is due to the low differences in their biodegradabilities, with respect to the original biodiesel. The experimental confidence interval is very close to the differences between the biodegradation curves for these mixtures and the lineal combination curve.

Table 4.3. Synergic factor (SF) of biodiesel on the biodegradation of fossil derived fuels

Crude Oil		Diesel		Gasoline	
% BD	SF	% BD	SF	% BD	SF
12.5	15.7	5.0	11.4	17.0	2.4
25.0	9.4	12.5	27.8	33.0	37.8
37.5	33.3	20.0	40.8	50.0	32.8
50.0	61.1	25.0	48.6	67.0	59.3
62.5	38.5	37.5	36.0	83.0	24.9
75.0	68.9	50.0	38.8		
87.5	-39.4	62.5	50.1		
		75.0	38.9		
		87.5	9.1		

Figures 4.10 to 4.12. show the comparisons of the lineal combination curves and the experimental results for all the mixtures.
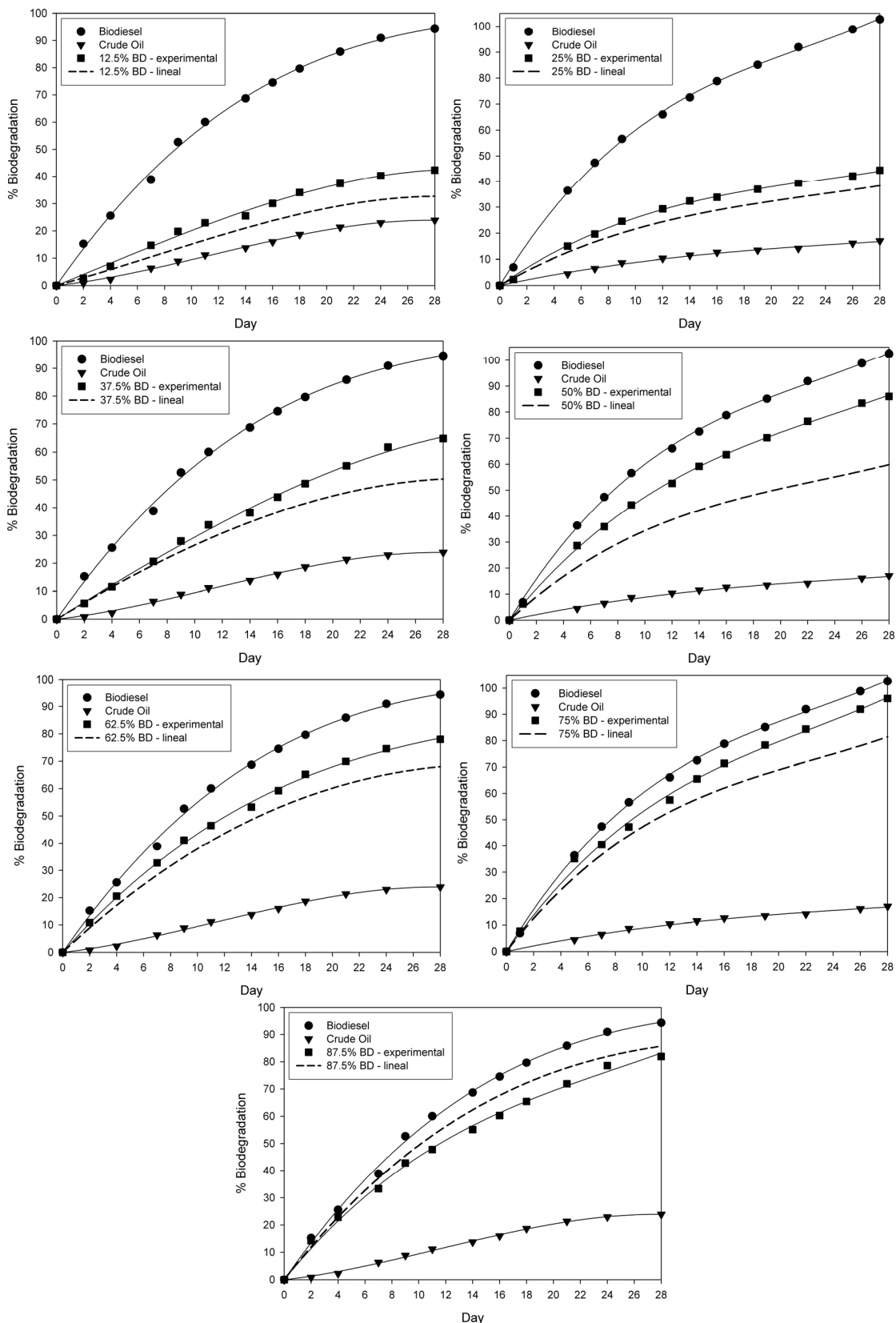
Figure 4.10. Synergic effects in the biodegradation of biodiesel-crude oil mixtures
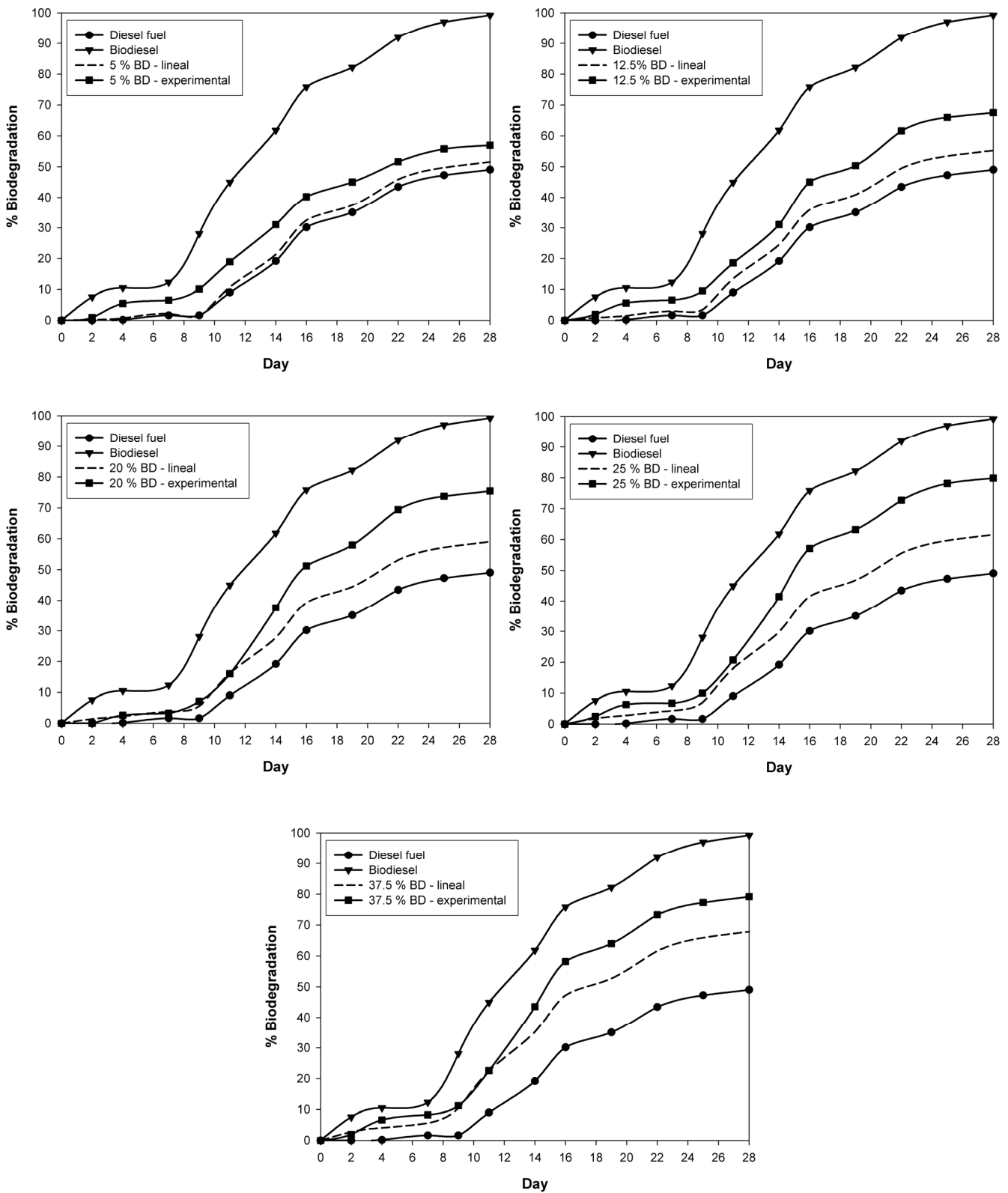
Figure 4.11 -a. Synergic effects in the biodegradation of biodiesel-diesel fuel mixtures
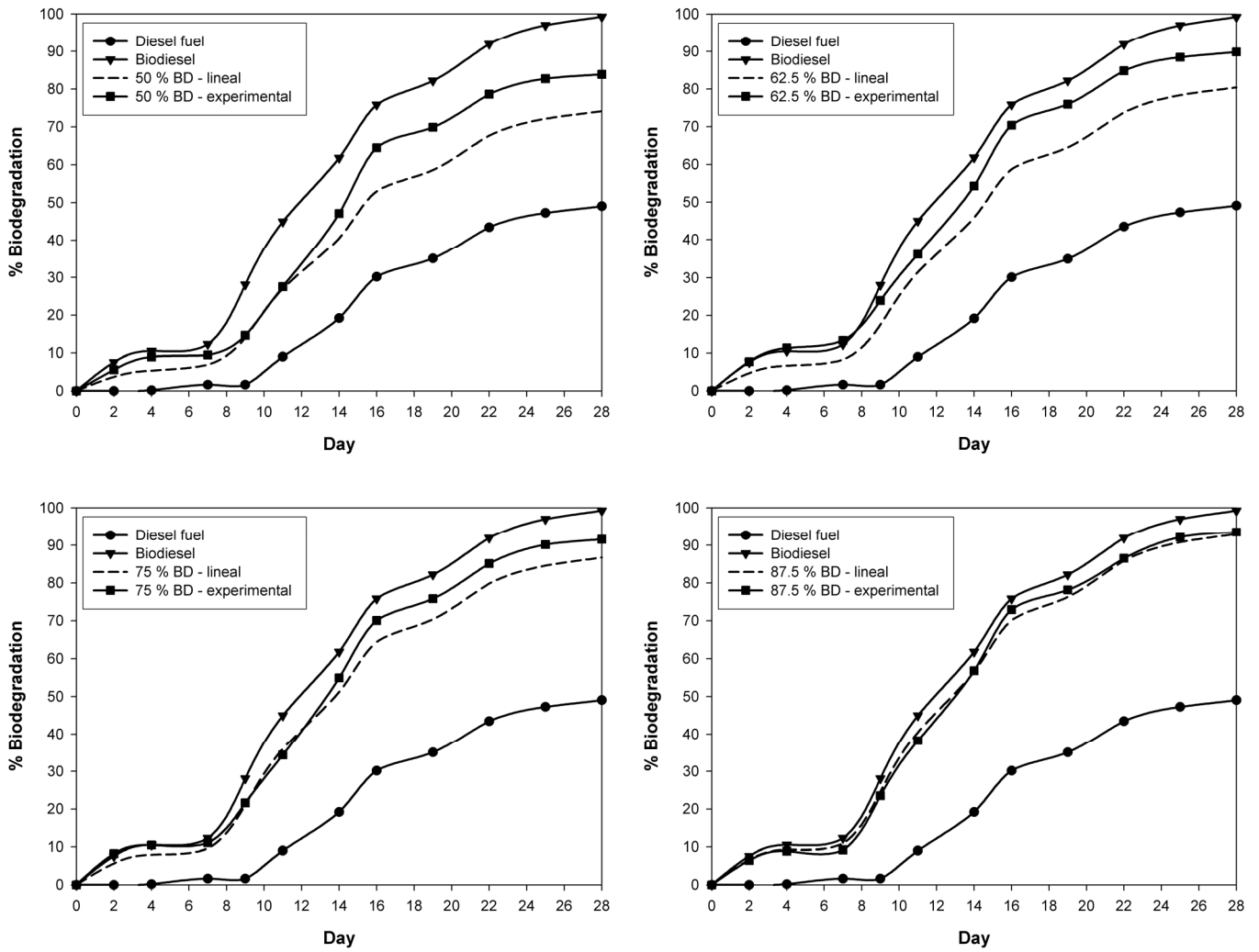
Figure 4.11 -b. Synergic effects in the biodegradation of biodiesel-diesel fuel mixtures
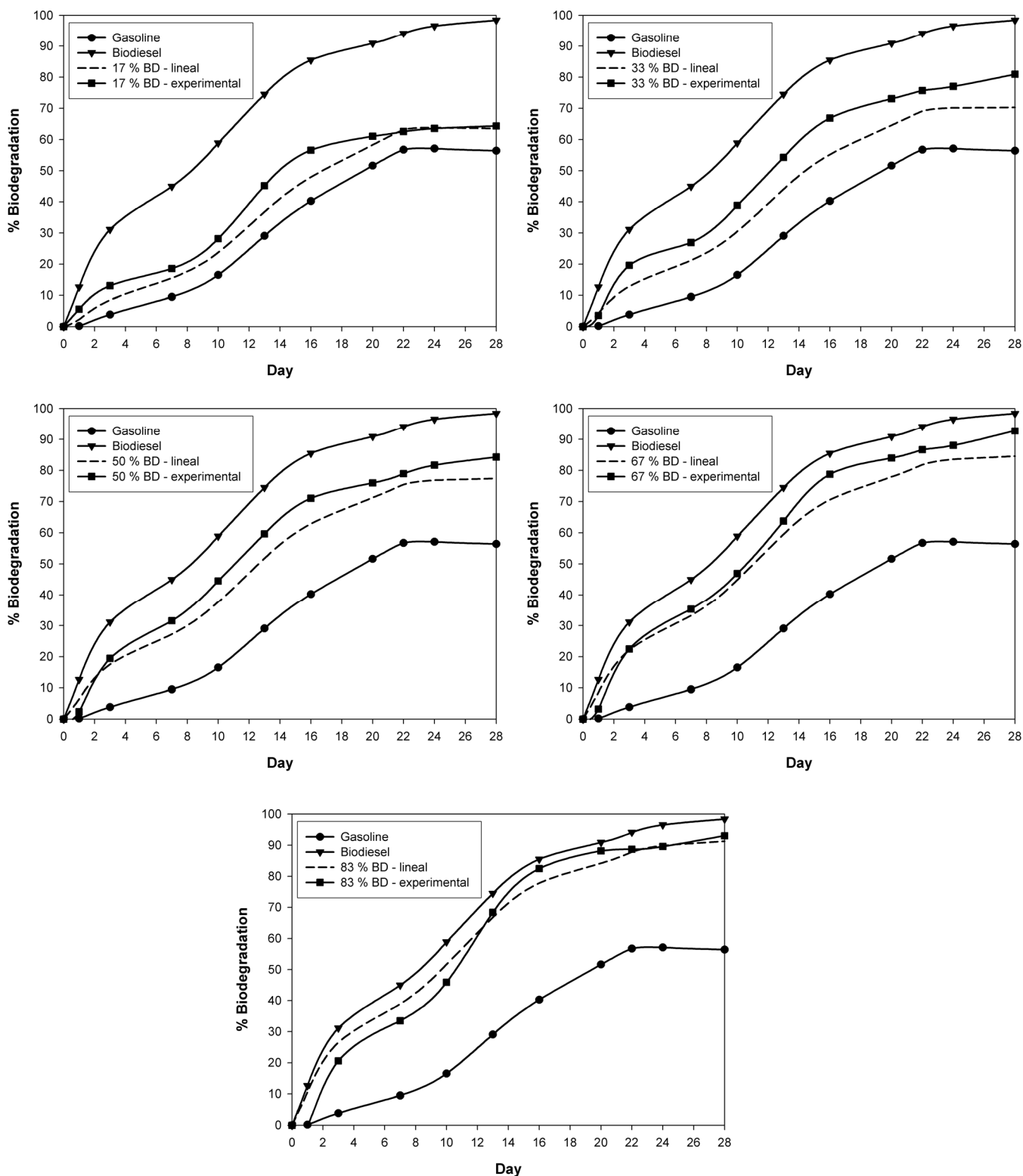
Figure 4.12. Synergic effects in the biodegradation of biodiesel-gasoline mixtures

4.4.2. PHYSICAL PROPERTIES

The density and viscosity of the different biodiesels is shown in table 4.4. All the values are among the limits established by European Normatives (EN 14214: Viscosity at 40 ºC = 3.50 – 5.00 mm^2/s, Density at 15 ºC = 860 – 900 Kg/m^3). For lower temperatures, the viscosity increases gradually.

Table 4.4. Density and viscosity of different types of biodiesel

Biodiesel	Density (Kg/m^3)			Viscosity (mm^2/s)		
	4 ºC	15 ºC	40 ºC	4 ºC	15 ºC	40 ºC
Used Oil	884.5	879.5	876.2	10.98	6.41	4.63
Cynara c.	886.4	879.1	878.1	9.87	6.22	4.16
Sunflower	887.1	878.1	786.3	9.02	6.42	3.58
Corn	884.5	877.5	876.9	9.93	6.47	4.04

Table 4.5 shows the viscosity of the mixtures of biodiesel and crude oil. The missing values correspond to samples whose viscosity is higher than the limit of the equipment used (10000 cp). At 4 ºC (the approximate temperature of the sea in the area of the Prestige oil spill) the viscosity could not be measured for the samples with the highest percentage of crude oil. However, the viscosity of the Prestige crude oil can be found in the bibliography *(CEDRE-b 2003)* for several temperatures, being approximately 300000 cp at 4 ºC. It is clear that the viscosity decreases with the addition of biodiesel, thus facilitating pumpability. For example, at 40 ºC, there is a change in viscosity of more than one order of magnitude, with only 12.5 % biodiesel.

Table 4.5. Viscosity of biodiesel-crude oil mixtures

Substrate	Viscosity (mm^2/s)		
	4 ºC	20 ºC	40 ºC
Biodiesel	8.7 ± 0.2[a]	4.8 ± 0.5	3.2 ± 0.2
12.5 % Fuel Oil	15.5 ± 0.2	10.2 ± 0.2	4.2 ± 0.4
25 % Fuel Oil	26 ± 0.5	17.1 ± 0.4	8.6 ± 0.4
37.5 % Fuel Oil	55 ± 0.6	24 ± 0.9	15.8 ± 0.7
50 % Fuel Oil	160 ± 3	24 ± 0.6	22 ± 0.8
62.5 % Fuel Oil	650 ± 12	185 ± 7	70 ± 2
75 % Fuel Oil	4900 ± 140	950 ± 25	200 ± 1
87.5 % Fuel Oil		4500 ± 50	260 ± 3
100 % Fuel Oil	300000[b]		7200 ± 200

[a] Confidence interval α=0.01
[b] Data from bibliography *(CEDRE-b 2003)*

The viscosity of the mixtures of biodiesel with diesel and gasoline is shown in figures 4.13 and 4.14. Both diesel and gasoline are less viscous than biodiesel and the viscosity of the mixtures increases gradually.
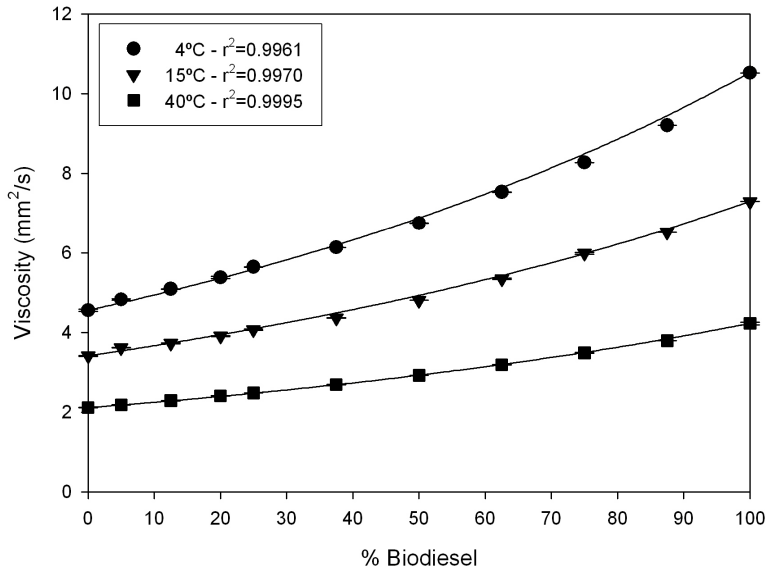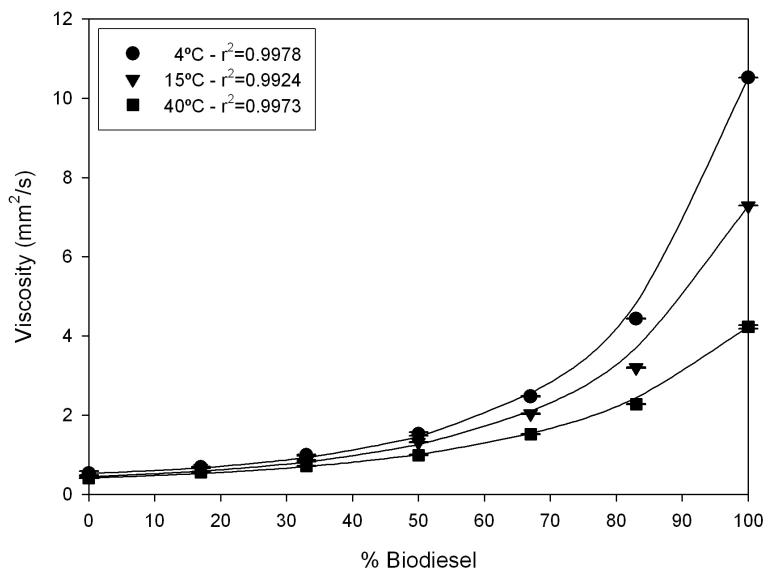

Figure 4.13. Viscosity of biodiesel-diesel fuel mixtures

Figure 4.14. Viscosity of biodiesel-gasoline fuel mixtures

The Grunberg and Nissan model for binary mixtures *(Perry 1984)* was used to adjust the viscosities of all the mixtures:

$$\mu_m = \exp\left(X_1 \ln \mu_1 + X_2 \ln \mu_2 + 2 X_1 X_2 \overline{G_{12}} \right) \tag{4.5}$$

where:

$$G_{12} = \ln \mu_m - X_1 \ln \mu_1 - X_2 \ln \mu_2 / (2 X_1 X_2) \tag{4.6}$$

$\overline{G_{12}}$: average value of the calculated G_{12}, and $\overline{G_{12}}$ =f(T).

X_1: mass fraction of component 1

X_2: mass fraction of component 2

μ_1: viscosity of component 1

μ_2: viscosity of component 2

μ_m: viscosity of the mixture

The $\overline{G_{12}}$ values obtained are shown in table 4.6.

Table 4.6. $\overline{G_{12}}$ Parameter for the viscosity of mixtures

Temperature (ºC)	Biodiesel - Diesel	Biodiesel – Gasoline
4	- 0.014	- 0.963
15	- 0.023	- 0.722
40	- 0.042	- 0.550

The change in density with the biodiesel content is shown in figures 4.15 to 4.17 for the mixtures with crude oil, diesel and gasoline respectively. The general trend shows that the addition of biodiesel decreases the density of crude oil and increases the density of diesel and gasoline. In all the cases, the density is lower than that of seawater, indicating that all the mixtures would not sink in the water.
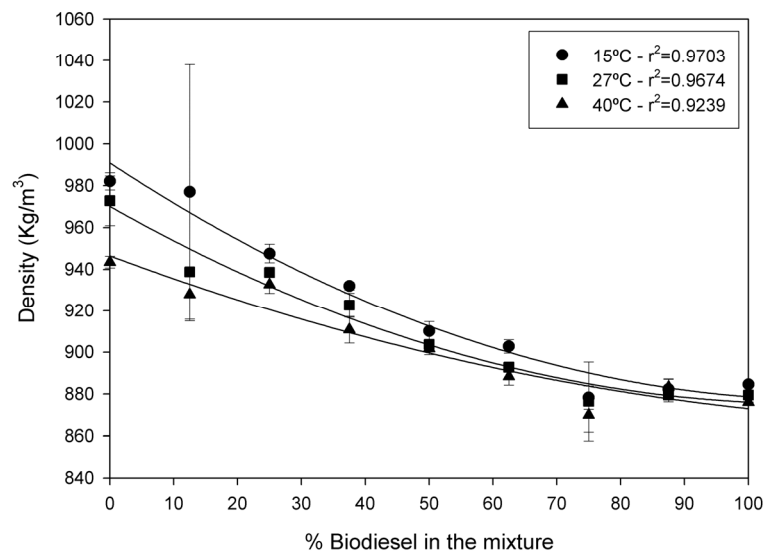
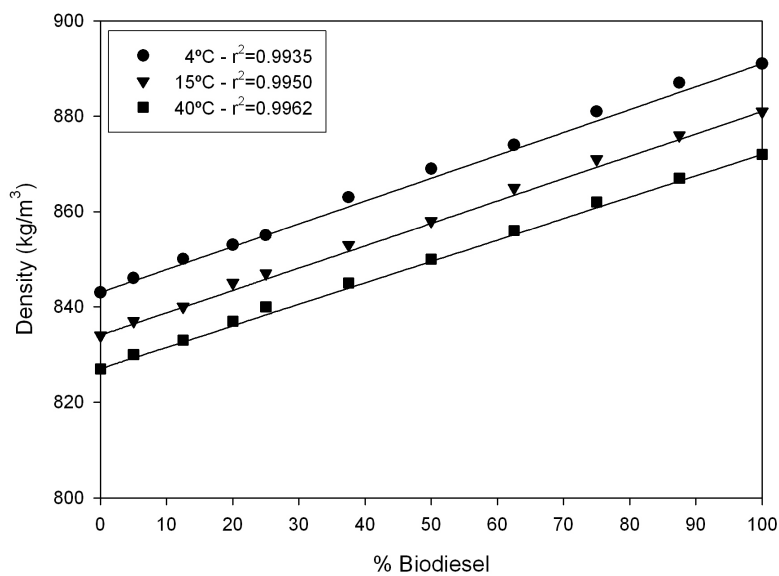Figure 4.15. Density of biodiesel-crude oil mixtures
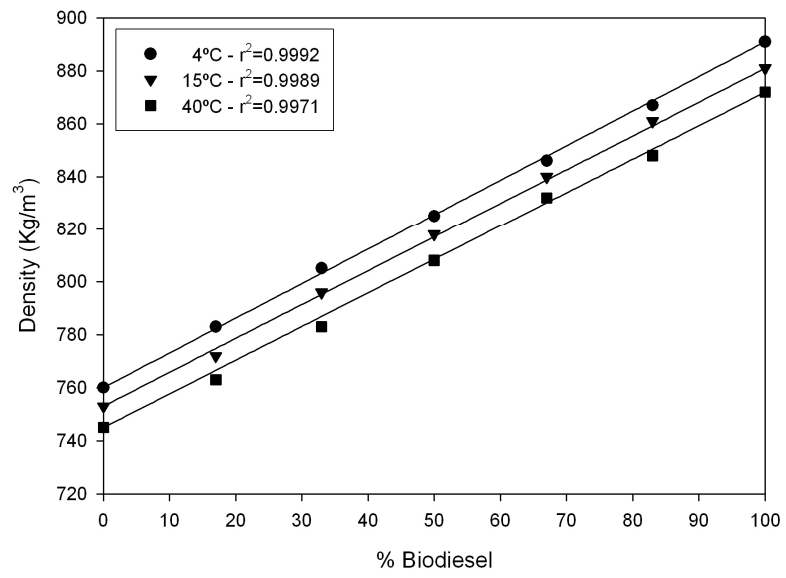

Figure 4.16. Density of biodiesel-diesel fuel mixtures

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Figure 4.17. Density of biodiesel-gasoline mixtures

4.4.3. POSSIBLE PRACTICAL APPLICATIONS

The former results can help the selection of appropriate design and operation conditions for in situ bioremediation. Although this technology must still be proved in the field, the results suggest that it could be put to the following environmentally-friendly uses:

Scenario 1: Fuel oil spill on the sea. The proposed solution is a biodiesel shower. This would decrease the density and keep the biodiesel -fuel oil mixture floating on the surface. The viscosity also decreases, thus making it easier to recover the mixture by means of a reciprocating pump. Once decanted, the water dragged with the mixture can be returned to the sea after irrigation of microorganisms and nutrients. As the biodegradability of the mixture is higher than that of the pure fuel oil, any remains of the mixture left in the sea or contained in the returned water would be degraded in a shorter time, with the help of the added microorganisms but also by means of the native ones. The recovered mixture containing the spilt fuel oil and the added biodiesel, can be burnt as fuel, for example, in a power plant, or in other more costly applications, such as low-speed diesel engines.

Scenario 2: Fuel oil on coast rocks. The proposed solution is a pressurised shower of biodiesel at high temperatures (80 ºC for example). The fuel oil embedded in the rocks is removed easily as it is dissolved in the biodiesel, and the mixture spills into the sea. The decrease in density keeps the mixture on the surface of the water, and the decrease in viscosity enables it to be pumped and recovered. The biodiesel -fuel oil mixture left on the surface of the rocks is easily biodegraded with the help of a shower of water containing microorganisms and nutrients. Also the water dragged with the mixture can be returned to the sea after irrigation of microorganisms and nutrients. The recovered mixture contains the spilt fuel oil and the added biodiesel, and can be burnt as fuel, for example, in a power plant, or in other more costly applications, such as low-speed diesel engines.

Scenario 3: "Lassagna" effect on a beach. After several "fuel tides", several layers of fuel and sand can build up on a beach. The proposed solution is to dig up the affected sand, and then shower it with biodiesel. The fuel oil embedded in the sand is dissolved by the biodiesel, its viscosity decreases and it can be easily dragged, thus facilitating the leaching and recovery of the mixture. Any remains of the mixture left in the sand can be easily biodegraded after bathing it with water containing microorganisms and nutrients, and continuously aerating the treated soil. It can then be returned to the sea. The recovered mixture contains the spilt fuel oil and the added biodiesel and can be burnt as fuel, for

example, in a power plant, or in other more costly applications, such as low-speed diesel engines. This treatment can also be applied to other kind of soils contaminated with different fossil fuels.

Other uses include the treatment of birds or other animals affected by an oil spill, as biodiesel has detergent properties and is non toxic.

The cost of any of these treatments will be compensated at least partially by the recovery of the biodiesel -fuel oil mixture, which that can be used as a fuel in several applications, the shorter time to restoration of the economic activity in the affected area, and, most important, the return to ecological normality of the treated place.

4.5. CONCLUSIONS

Biodiesel has shown to easily dissolve crude oil, diesel and gasoline, for all the different mixture proportions. The change in the physical properties measured (density and viscosity) due to the mixture with biodiesel allows the design of possible remediation applications in the field. Because of its density, pure crude oil can float on the surface of seawater. The addition of biodiesel decreases the density, showing that the mixtures will float even better. The viscosity is a very important parameter, because crude oil has very high values that makes it difficult to recover and handle by pumping. This viscosity can be decreased in several orders of magnitude with the addition of relatively low amounts of biodiesel. Although the addition of biodiesel increases the viscosity of diesel and gasoline, it is always low enough to allow pumping.

The residual biodiesel-fossil derived fuel mixture in the treated area, after the recovery will be easily degraded in a short time as confirmed in the biodegradability experiments performed. In all the cases the biodegradation of crude oil, diesel and gasoline was enhanced by the addition of biodiesel, showing the effects of cometabolism.

For practical applications, the percentage of biodiesel added to the affected area should be as low as possible to reduce the cost of the operation and the volume of the recovered product. The best mixture is the one that has a low percentage of biodiesel, a low viscosity, a high biodegradability and a high synergic effect. In the case of crude oil, the mixture with 50% biodiesel seems to be the most adequate one, as it considerably increases the biodegradability of the crude oil, reaching a biodegradation of 85% in only one month, with a synergic effect of 61%. For all the temperatures tested, the viscosity of this mixture is also several orders of magnitude lower than that of pure crude oil.

In diesel fuel or gasoline spills in aquatic environments, it is extremely difficult to physically recover all the fuel, and some of it is always left in the area. Adding biodiesel to recover what remains of the fossil fuel seems to be a good solution in the bioremediation of the affected site. The remaining biodiesel-diesel fuel or biodiesel-gasoline mixture in the treated area after the physical recovery will be easily degraded in a short time, as our biodegradability experiments confirmed. In all the cases, the biodegradation of the fossil-derived fuels was enhanced by adding biodiesel, due to the effects of cometabolism.

In the case of diesel fuel, the mixture with 50% biodiesel reaches a biodegradation of 83% in only one month. However, with lower amounts of biodiesel like 25-37.5%, the results are also good, reaching almost 80% biodegradation with synergic effects of 48 and 36% respectively. As higher amounts of biodiesel do not considerably increase the synergic effect, the addition of 25% biodiesel to the diesel fuel spilt would be good enough for bioremediation. In the case of gasoline, the mixture with 67% biodiesel reaches a biodegradation of nearly 93% in only one month and presents the highest synergic factor (almost 60%). However, with lower amounts of biodiesel like 33%, the results are also good, reaching almost 81% biodegradation with a synergic effect of 38%.

The results for the mixtures of biodiesel and fossil diesel fuel acquire greater importance if it is borne in mind that biodiesel-diesel fuel blends are commercially available at fuel stations and transport companies in several countries. Much of the biodiesel produced in the world is consumed in mixtures with diesel fuel, specially the common blends known as B-5 and B-20 (5 and 20% biodiesel respectively), and the risks of spillage are the same as those of diesel fuel. The time needed to reach 50% biodegradation is reduced from 28 to 22 days in the case of B-5 and from 28 to 16 days in the case of B-20, at ambient temperatures. The synergic effect of biodiesel on the biodegradation of fossil diesel fuel and the reduction of emissions are further ecological advantages of this renewable fuel.

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GENERAL CONCLUSIONS

The main conclusions of this work are that:

➢ The reaction conditions where optimised for the transesterification of unrefined Cynara cardunculus oil, with and without pre-treatment steps

➢ The pre-treatment steps are necessary to increase the ester yield of the reaction and to obtain a product with an acceptable quality

➢ It was demonstrated the need for specific analytical methods and standard requirements for the mixtures of biodiesel and diesel-fuel

➢ Biodiesel can be applied to cometabolise the biodegradation of some fossil derived fuels, such as crude oil, diesel fuel and gasoline

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FUTURE WORK

As a result of the present work, other subjects appear as a possible continuation and complement of what was done. The transesterification reaction could be improved if other refining steps are applied to the oil in order to remove unsaponifiable compounds. The results from the reaction optimisation could be useful to design and operate a pilot plant.

It is also necessary to develop analytical methods specific for the mixtures of biodiesel and diesel fuel, in those cases that require them. Specific limits for the quality requirements are also needed for the mixtures.

It could also be interesting to study the effects of biodiesel in the biodegradability of fossil derived fuels in soils.

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APPENDIX

APPENDIX A – GC DETERMINATION OF BIODIESEL COMPOSITION

Biodiesel composition was determined using two Agilent 6850 gas chromatographs (Figure A.1). GC1 (left chromatograph) was equipped with an on-column inlet and a FID detector. The column used was a DB-5 (5%-phenyl-methylpolysiloxane phase), a capillary non-polar column, with a high temperature limit and dimensions 15 m x 0.32 mm x 0.10 μm. This GC equipment was used for the analysis of the mono-, di-, triglycerides and glycerol, according to EN 14105. GC2 (right chromatograph) was equipped with a split/splitless inlet and a FID detector. The column used was a HP-INNOWax (polyethylene glycol phase), a capillary column with a high polarity and dimensions 30 m x 0.32 mm x 0.25 μm. This GC equipment was used for the analysis of methyl esters composition and total content, according to EN 14103.

Figure A.1. Gas chromatographs

EN 14105 TEST METHOD

Two internal standards were used for calibration and sample analysis. The internal standard for the determination of glycerol was 1,2,4 butanetriol (IS1) (1 mg/ml pyridine solution) and the internal standard for the determination of glycerides was 1,2,3 tricaproylglycerol (IS2) (8 mg/ml pyridine solution). Calibration patrons were glycerol (0.5 mg/ml pyridine solution), monoolein, diolein and triolein (5 mg/ml pyridine solution, each).

Four calibration solutions were prepared using different amounts of the four patrons and the two internal standards. The solutions were sililated with the addition of 100 µl of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA). After 15 minutes, 8 ml heptane were added and 1 µl solution was injected to GC1. Solutions prepared in this way, are stable for 24 hours.

Biodiesel samples were prepared by weighting 100-200 mg biodiesel, and adding 80 µl of IS1 and 100 µl of IS2. The samples were also sililated with MSTFA and left to react for 15 minutes before the addition of 8 ml heptane. Then, 1 µl solution was injected to GC1. Samples prepared in this way, are stable for 24 hours.

Figures A.2. to A.5 show some examples of chromatograms for a calibration solution, *Cynara cardunculus* oil, pre-treated oil and biodiesel.
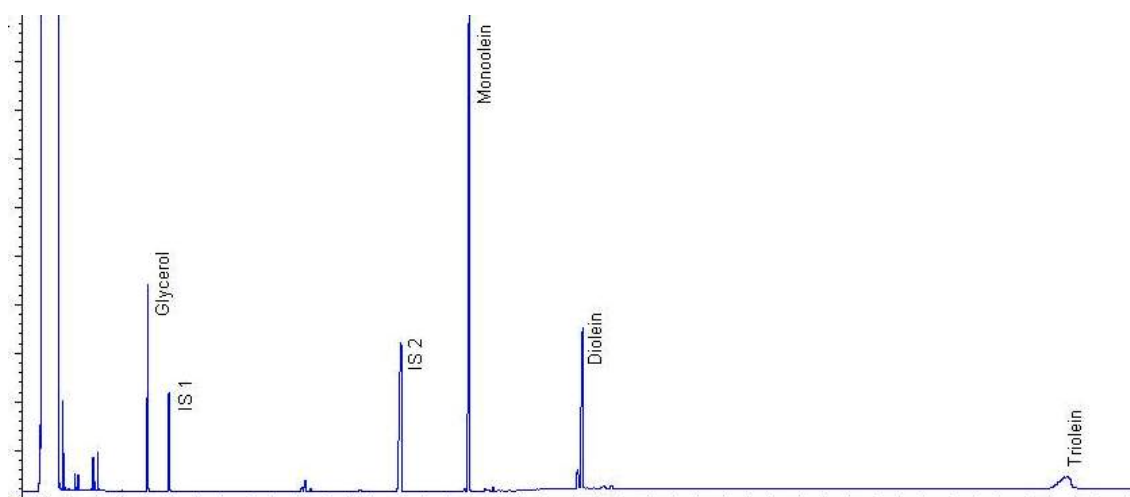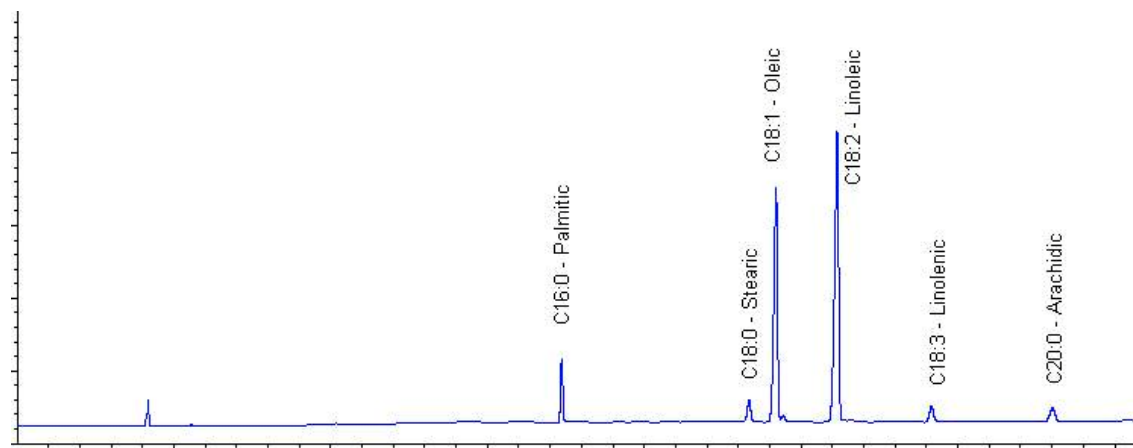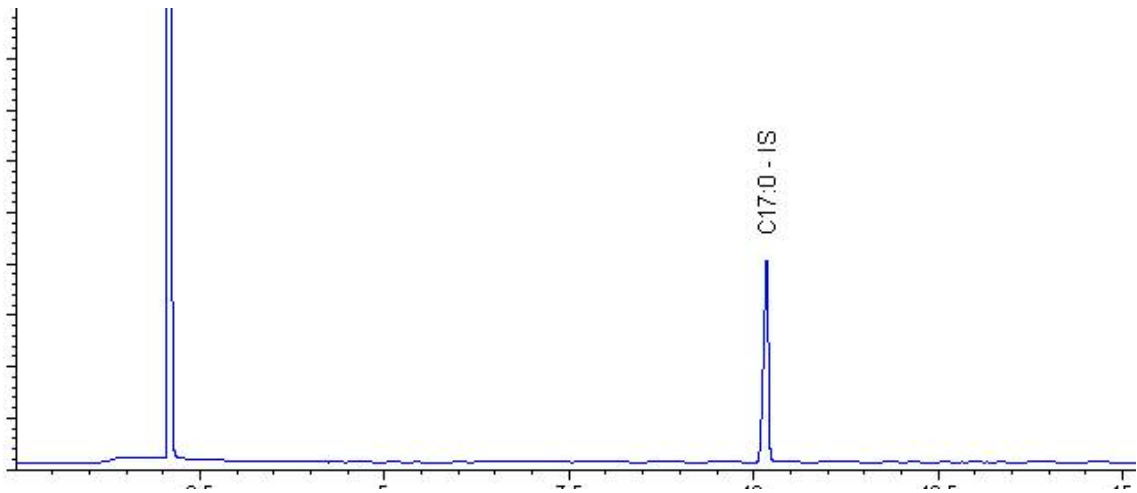

Figure A.2. Example of chromatogram (GC1) for a calibration solution.
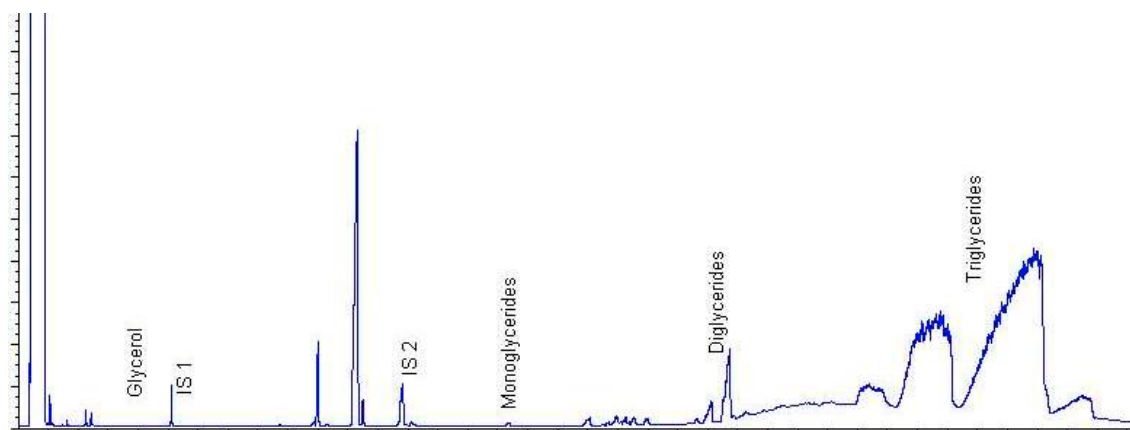
Figure A.3. Example of chromatogram (GC1) for unrefined *Cynara cardunculus* oil.

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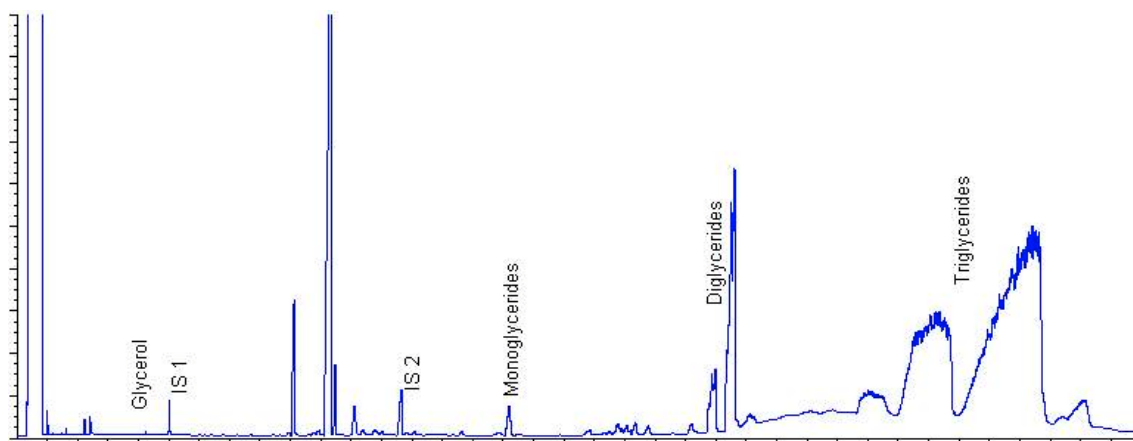
Figure A.4. Example of chromatogram (GC1) for preesterified *Cynara cardunculus* oil.
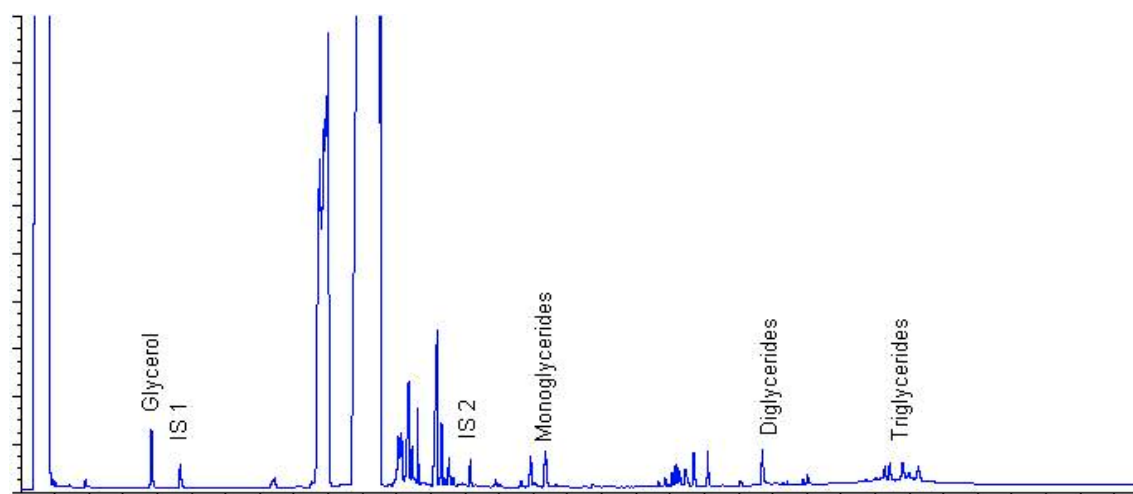
Figure A.5. Example of chromatogram (GC1) for biodiesel from pre-treated *Cynara cardunculus* oil.

EN 14103 TEST METHOD

Methyl heptadecanoate (C17:0) was used as internal standard for calibration and sample analysis, in a 10 mg/ml heptane solution. The calibration patrons were three mixtures of pure fatty acid methyl esters, with a known and certified ester composition, obtained from SUPELCO. FAME-MIX-1 contained palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) methyl esters, at the same proportions found in sunflower oil. FAME-MIX-2 contained myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosenic (20:1), behenic (C22:0), erucic (22:1) and lignoceric (C24:0) methyl esters, at the same proportions found in rapeseed oil. FAME-MIX-3 contained a mixture of methyl esters from C8 to C24.

Biodiesel samples were prepared by adding 1 ml of the methyl heptadecaniate solution and 4 ml heptane. Then, 3 μl solution were injected to GC2.

Figures A.6. to A.9 show some examples of chromatograms for a calibration solution, *Cynara carduncul*us oil, pre-treated oil and biodiesel.

Figure A.6. Example of chromatogram (GC2) for FAME-MIX-1.

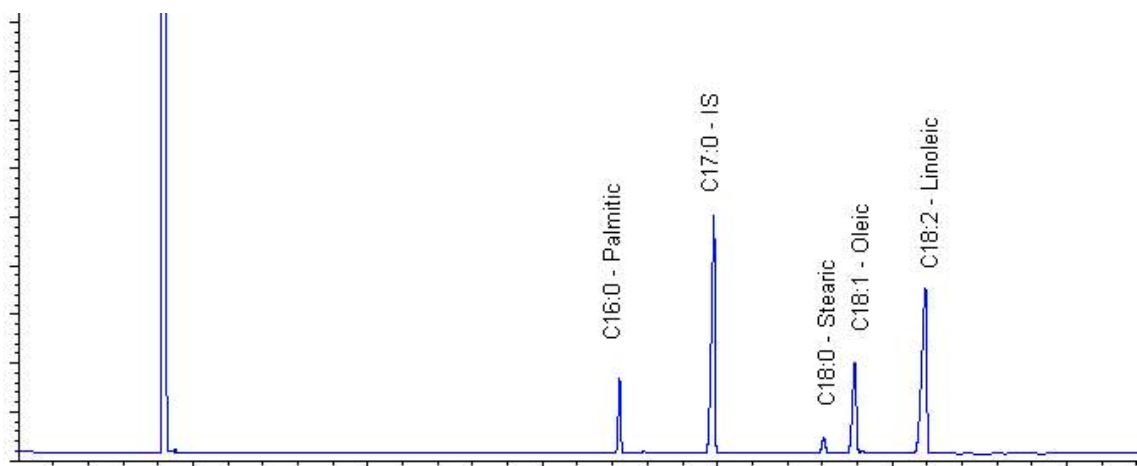Figure A.7. Example of chromatogram (GC2) for unrefined *Cynara cardunculus* oil.

Figure A.8. Example of chromatogram (GC2) for preesterified *Cynara cardunculus* oil.
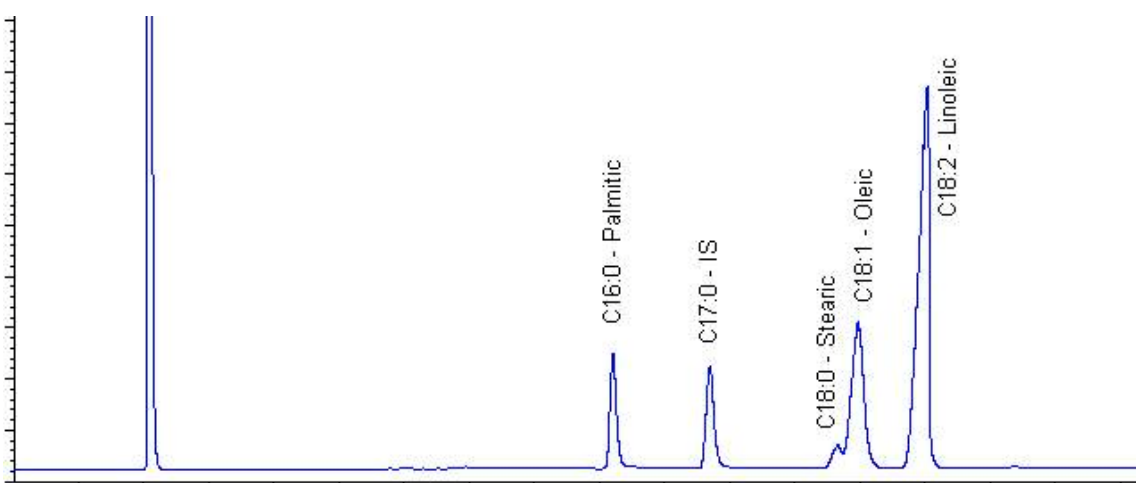

Figure A.9. Example of chromatogram (GC2) for biodiesel from pre-treated *Cynara cardunculus* oil.

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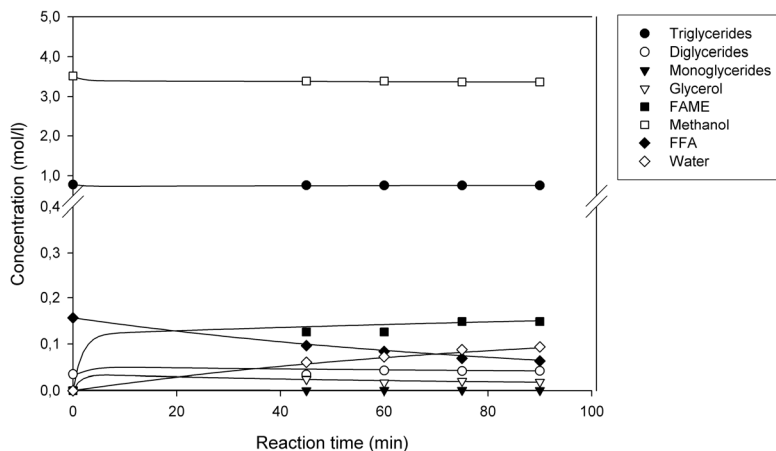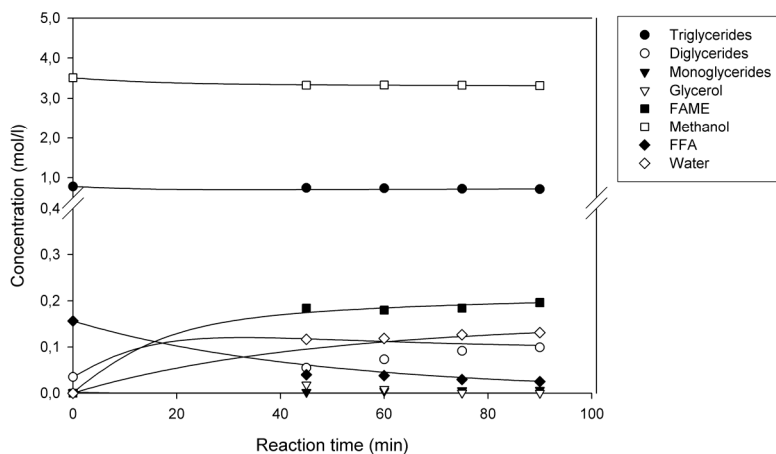
APPENDIX B - KINETICS OF THE PREESTERIFICATION REACTION

Figure B.1. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40°C using 4.5:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.
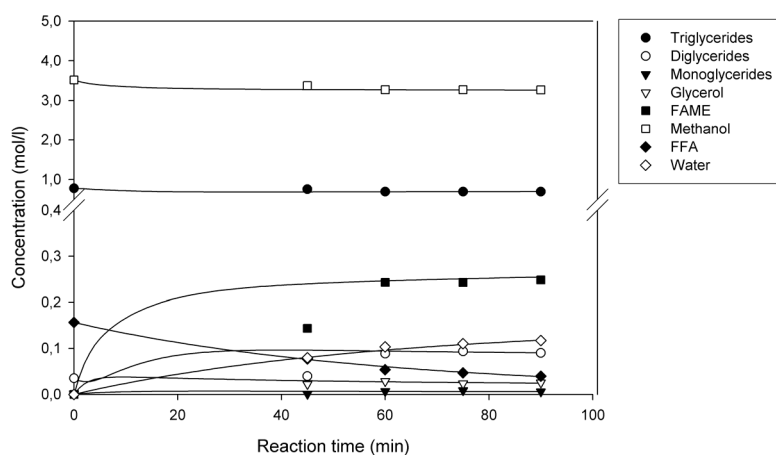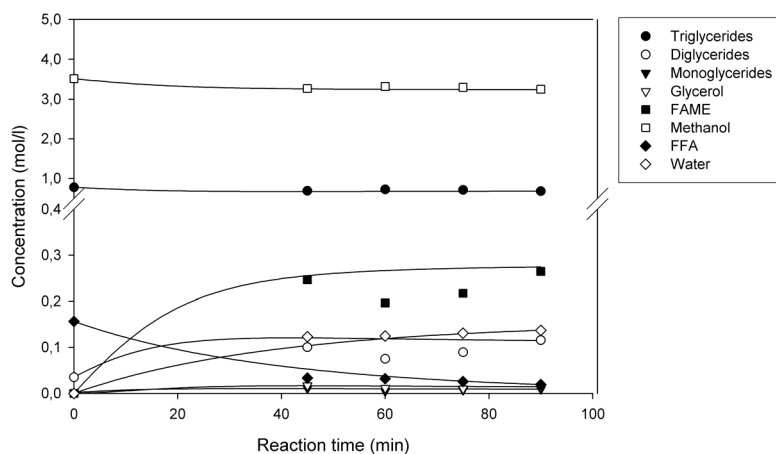

Figure B.2. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60°C using 4.5:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.

Table B.1. Kinetic parameters for the reaction with 4.5:1 MeOH ratio and 0.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40°C	60°C	E_a (J/mol)	A (l/mol.min)
k_1	0.0077	0.0023	-761	1.30×10^{-11}
k_2	3.15	0.28	-1522	8.90×10^{-18}
k_3	0.24	0.039	-1127	2.39×10^{-14}
k_4	401	9.71	-2333	4.97×10^{-25}
k_5	4.46	0.08	-2523	3.57×10^{-29}
k_6	3.1	53	1787	$1.24 \times 10^{+21}$
k_7	0.003	0.0062	455	535
k_8	0.004	0.0018	-499	7.04×10^{-9}

Figure B.3. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 4.5:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.
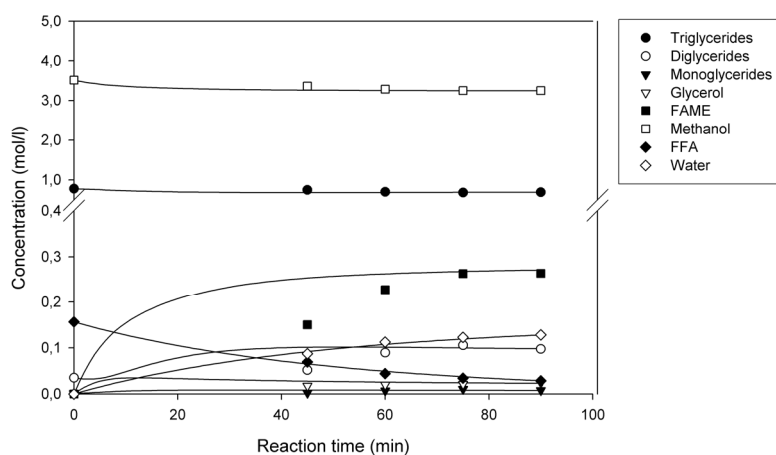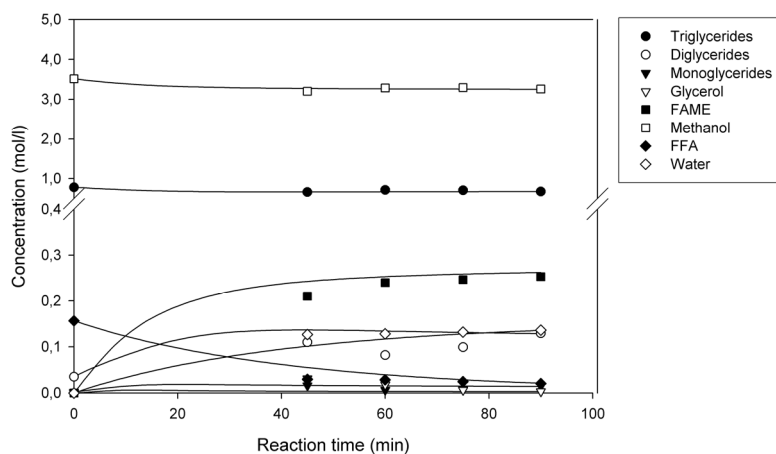

Figure B.4. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 4.5:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.

Table B.2. Kinetic parameters for the reaction with 4.5:1 MeOH ratio and 1.0% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.003	0.0026	-89	2.78×10^{-4}
k_2	0.31	0.19	-306	9.08×10^{-5}
k_3	0.12	0.007	-1818	1.35×10^{-22}
k_4	24.6	1.06	-1973	4.41×10^{-22}
k_5	2.5	0.034	-2683	2.86×10^{-31}
k_6	7.7	0.27	-2102	4.45×10^{-24}
k_7	0.0049	0.0073	250	3.75
k_8	0.002	0.0012	331	3.13×10^{-7}

Figure B.5. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 4.5:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.
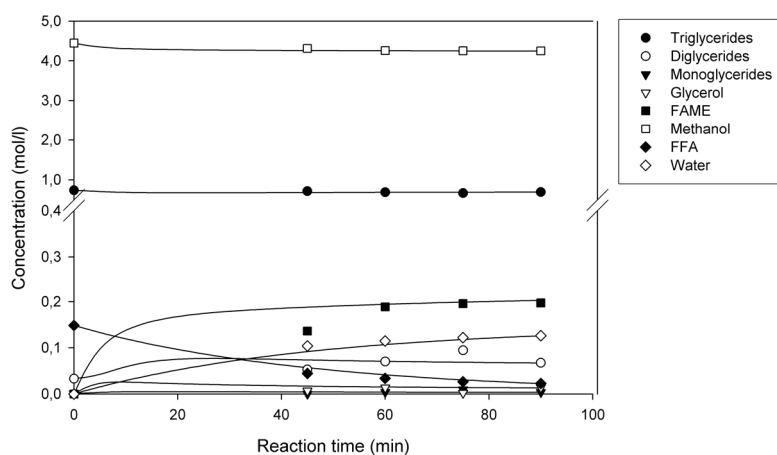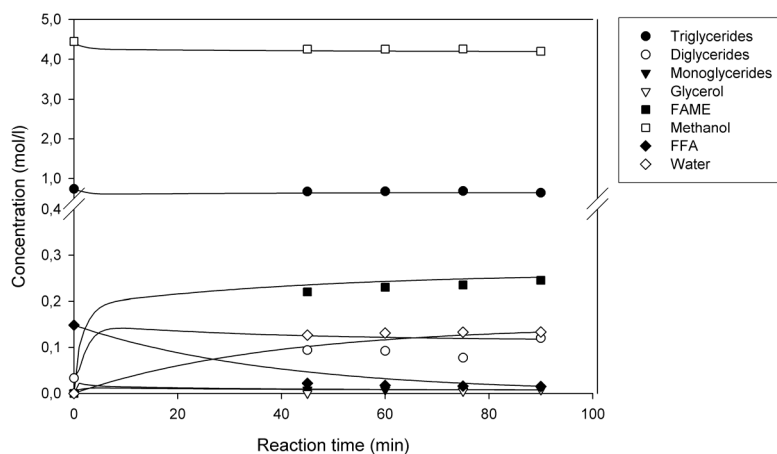

Figure B.6. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 4.5:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.

Table B.3. Kinetic parameters for the reaction with 4.5:1 MeOH ratio and 1.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.0023	0.0029	145	0.11
k_2	0.2	0.193	-21	0.12
k_3	0.066	0.015	-917	1.75×10^{-12}
k_4	10.5	1.76	-1117	1.37×10^{-12}
k_5	1.29	0.05	-2039	4×10^{-24}
k_6	5.05	3.11	302	1.6×10^{-3}
k_7	0.006	0.007	102	0.09
k_8	0.0015	0.0012	-120	6.34×10^{-5}

Figure B.7. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 6:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.
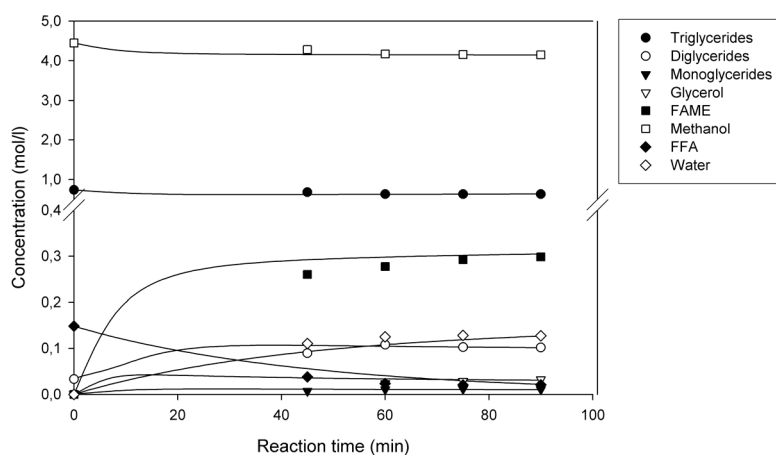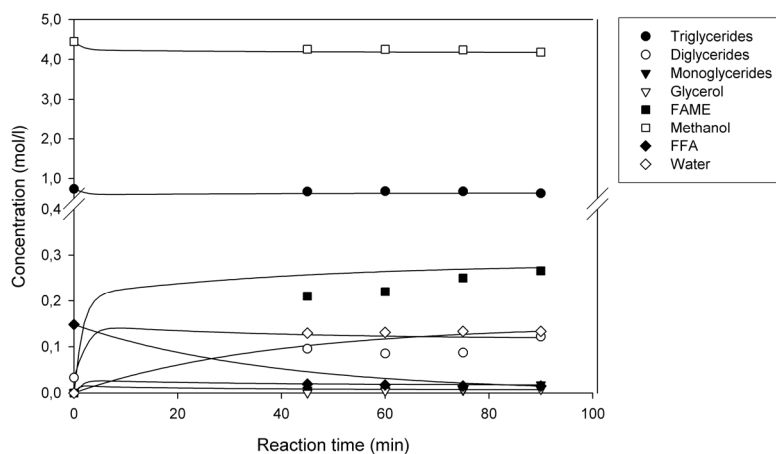

Figure B.8. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.

Table B.4. Kinetic parameters for the reaction with 6:1 MeOH ratio and 1.0% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.0025	0.016	1165	$6.71 \times 10^{+10}$
k_2	0.55	1.46	610	$6.01 \times 10^{+6}$
k_3	0.046	1.33	2110	$9.82 \times 10^{+22}$
k_4	18	351	1859	$4.92 \times 10^{+22}$
k_5	0.97	2.4	569	$3.55 \times 10^{+6}$
k_6	5.6	40	1230	$8.65 \times 10^{+14}$
k_7	0.005	0.0061	125	0.14
k_8	0.001	0.00068	-284	5.6×10^{-7}

Figure B.9. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 6:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.
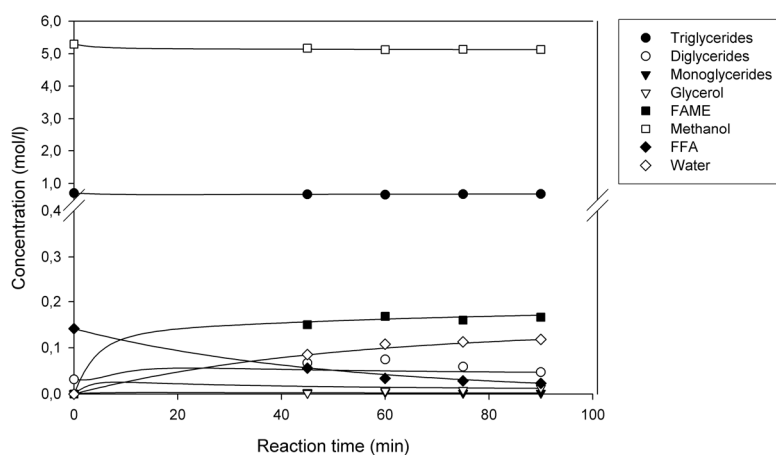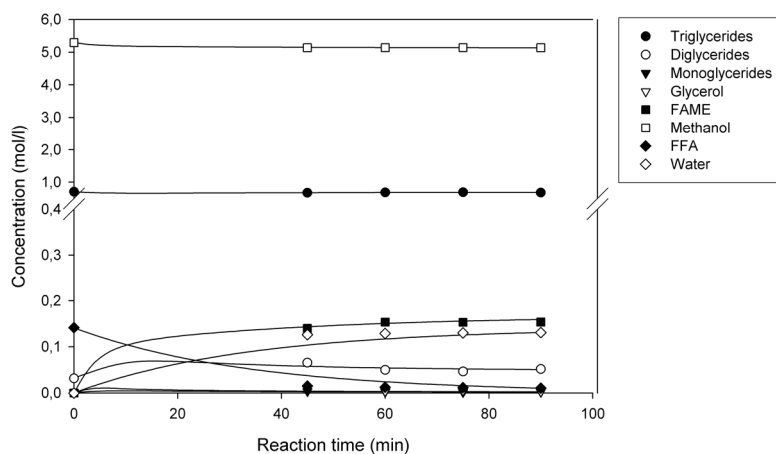

Figure B.10. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 6:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.

Table B.5. Kinetic parameters for the reaction with 6:1 MeOH ratio and 1.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.0033	0.019	1098	$1.52 \times 10^{+10}$
k_2	0.28	1.54	1059	$4.64 \times 10^{+11}$
k_3	0.041	0.10	585	$2.28 \times 10^{+5}$
k_4	5.65	11	419	$3.82 \times 10^{+5}$
k_5	0.68	2.14	719	$1.33 \times 10^{+8}$
k_6	3.05	81	2057	$1.60 \times 10^{+24}$
k_7	0.0052	0.0062	110	0.097
k_8	0.0007	0.00062	-67	1.12×10^{-4}

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Figure B.11. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 7.5:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.
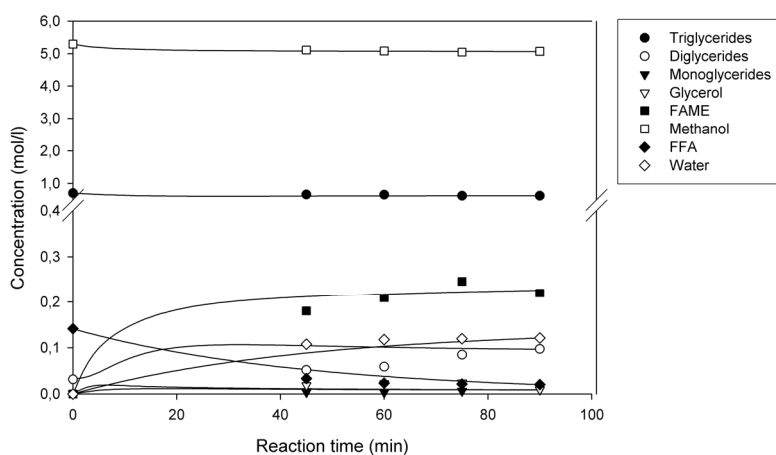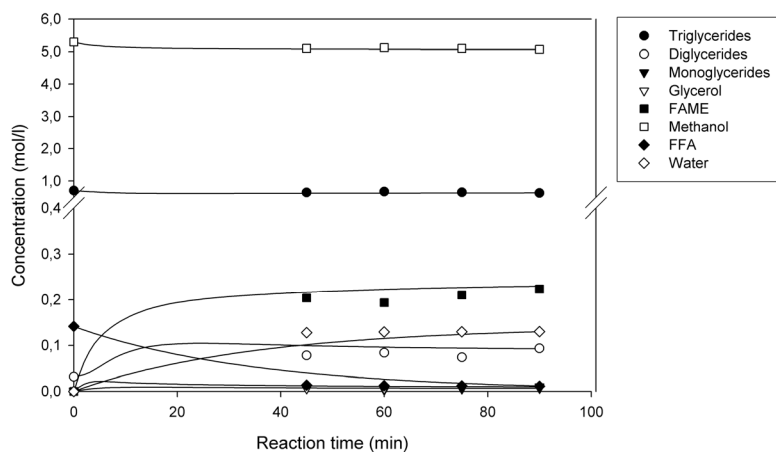

Figure B.12. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 7.5:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.

Table B.6. Kinetic parameters for the reaction with 7.5:1 MeOH ratio and 0.5% H_2SO_4.

|	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.002	0.0024	114	0.04
k_2	0.88	1.05	110	16.2
k_3	0.054	0.023	-548	2.59×10^{-8}
k_4	38.6	17.5	-498	7.03×10^{-5}
k_5	1.0	0.40	-576	2.27×10^{-7}
k_6	4.96	10.4	464	$1.10 \times 10^{+6}$
k_7	0.004	0.0057	230	1.77
k_8	0.0009	0.0006	274	6.29×10^{-7}

Figure B.13. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 7.5:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.
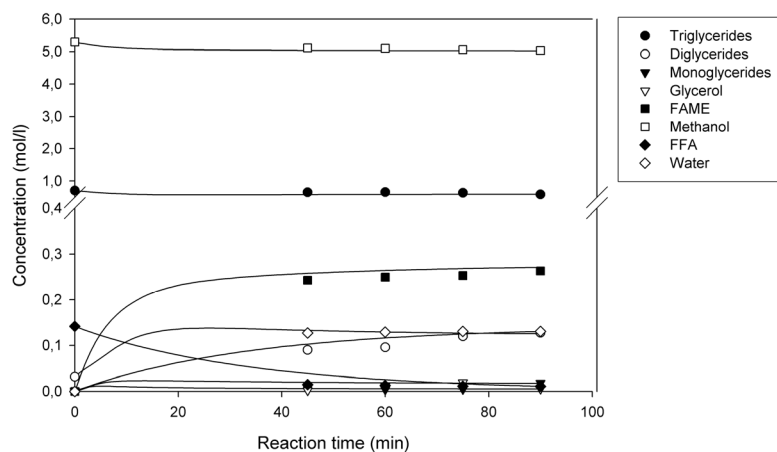

Figure B.14. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 7.5:1 methanol molar ratio and 1.0% H_2SO_4 as catalyst.

Table B.7. Kinetic parameters for the reaction with 7.5:1 MeOH ratio and 1.0% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.0025	0.0032	155	0.15
k_2	0.37	0.49	176	39
k_3	0.043	0.06	202	9.26
k_4	10.9	18.7	342	$9.62 \times 10^{+4}$
k_5	0.79	1.05	183	101
k_6	17	15.5	-59	3.52
k_7	0.0043	0.0055	157	0.28
k_8	0.00064	0.00043	-253	7.83×10^{-7}

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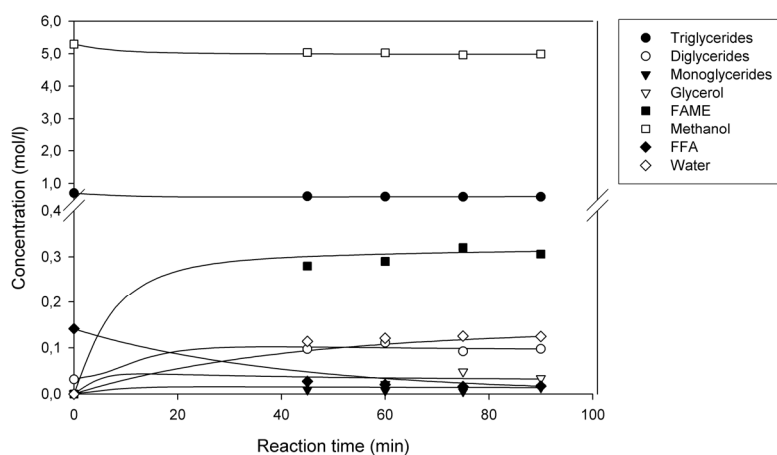
Figure B.15. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 7.5:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.
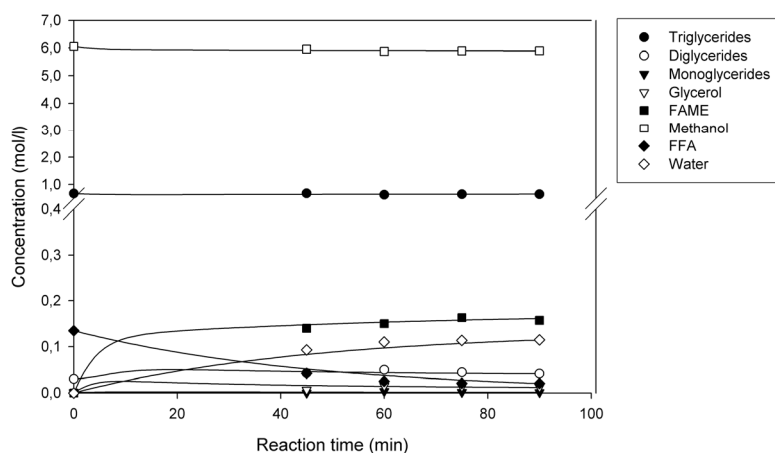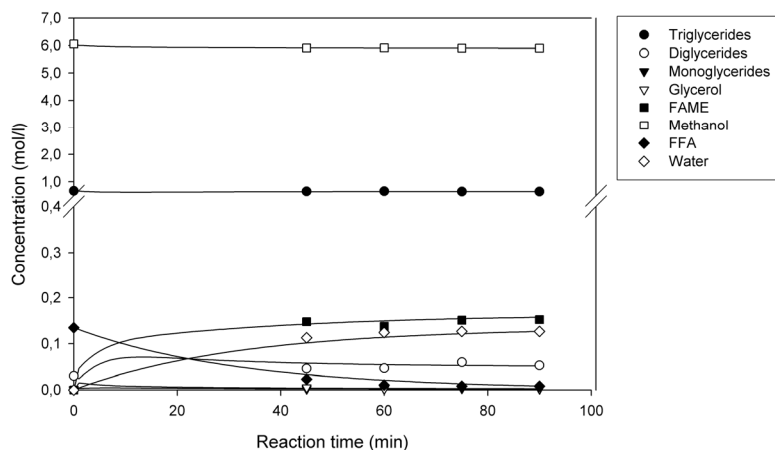

Figure B.16. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 7.5:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.

Table B.8. Kinetic parameters for the reaction with 7.5:1 MeOH ratio and 1.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.003	0.0043	225	1.21
k_2	0.30	0.38	154	17.8
k_3	0.048	0.032	-244	7.35×10^{-5}
k_4	5.5	4.36	-144	0.12
k_5	0.79	0.68	-91	0.07
k_6	5.33	43	1313	$7.37 \times 10^{+15}$
k_7	0.0048	0.0057	108	0.084
k_8	0.00042	0.00036	100	2.96×10^{-5}

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Figure B.17. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 9:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.
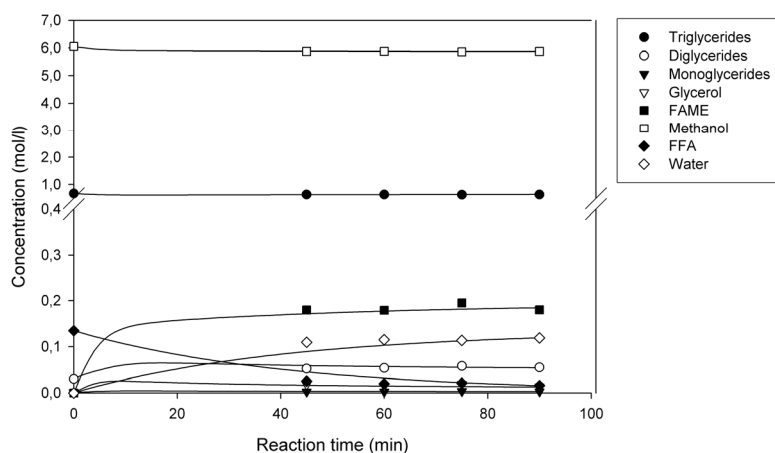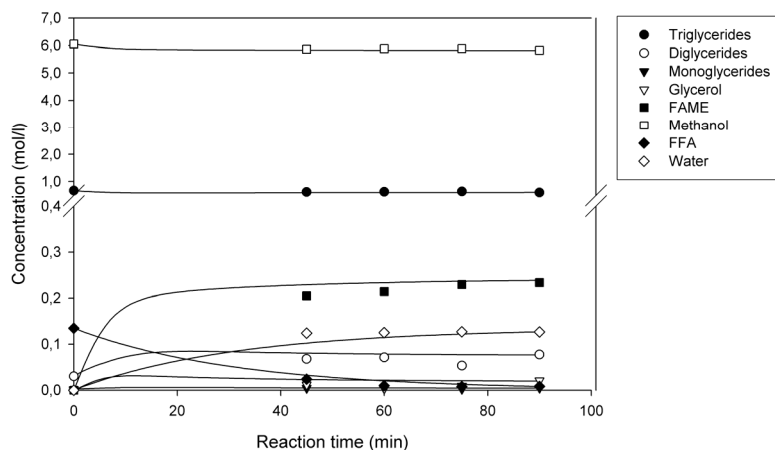

Figure B.18. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 9:1 methanol molar ratio and 0.5 % H_2SO_4 as catalyst.

Table B.9. Kinetic parameters for the reaction with 9:1 MeOH ratio and 0.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.002	0.003	233	0.975
k_2	1.17	1.37	100	16.7
k_3	0.041	0.58	1668	$7.03 \times 10^{+17}$
k_4	44	560	1591	$9.77 \times 10^{+19}$
k_5	0.79	1.45	384	$2.09 \times 10^{+4}$
k_6	3.44	40	1545	$2.28 \times 10^{+18}$
k_7	0.0036	0.0053	238	1.99
k_8	0.0007	0.0004	-360	4.82×10^{-8}

Figure B.19. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 9:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.
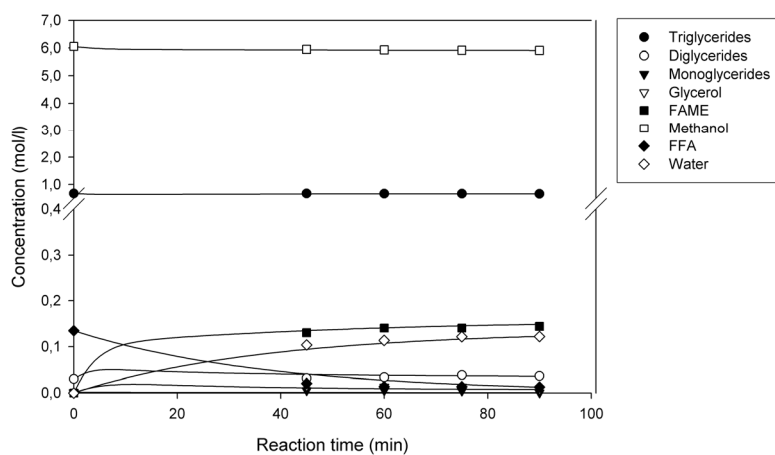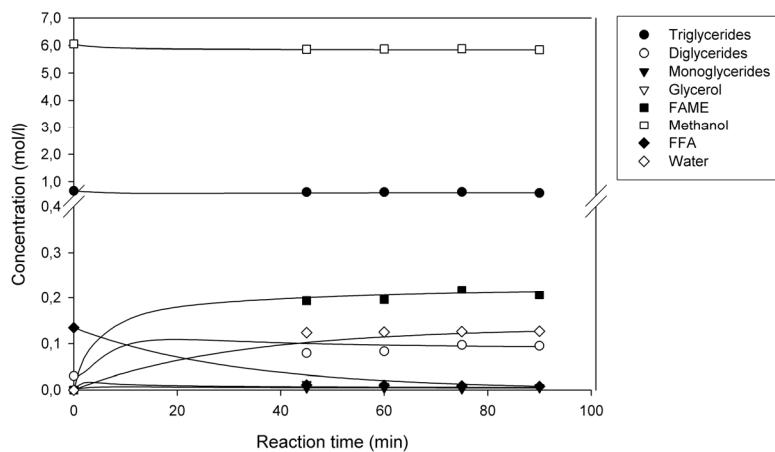

Figure B.20. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 9:1 methanol molar ratio and 1.0 % H_2SO_4 as catalyst.

Table B.10. Kinetic parameters for the reaction with 9:1 MeOH ratio and 1.0% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.003	0.0032	40	0.09
k_2	1.12	0.62	-371	5.91×10^{-5}
k_3	0.032	0.031	-27	0.016
k_4	20	13	-269	0.016
k_5	0.61	0.33	-390	1.94×10^{-5}
k_6	4.3	1.8	-544	2.26×10^{-6}
k_7	0.0041	0.0054	173	0.4
k_8	0.0005	0.0003	-432	5.31×10^{-9}

Figure B.21. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 40ºC using 9:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.

Figure B.22. Kinetic modelling curves (lines) and experimental data (points) for the reaction conducted at 60ºC using 9:1 methanol molar ratio and 1.5 % H_2SO_4 as catalyst.

Table B.11. Kinetic parameters for the reaction with 9:1 MeOH ratio and 1.5% H_2SO_4.

	Specific rate constant (l/mol.min)		Arrhenius parameters	
	40ºC	60ºC	E_a (J/mol)	A (l/mol.min)
k_1	0.0028	0.0035	140	0.115
k_2	2.07	0.62	-754	4.16×10^{-9}
k_3	0.024	0.084	786	$2.97 \times 10^{+7}$
k_4	55.8	48.9	-82	6.34
k_5	0.44	0.85	408	2.24×10^4
k_6	1.50	18.8	1583	$2.73 \times 10^{+18}$
k_7	0.0045	0.0054	114	0.094
k_8	0.0005	0.0003	-416	8.72×10^{-9}

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Appendix C – PROCEDURE FOR KINETIC ANALYSIS WITH GEPASI PROGRAM

GEPASI's main interface (figure C.1) gives a general view of the reaction parameters that can be defined to the program, as the number of reaction, the reactants present (metabolites), the type of kinetics, units employed, links between variables, etc.

The procedure to solve a problem with GEPASI is the following:

- ➢ Define the reaction mechanism (figure C.2).). The reactions added must be defined as a sum of reactants and products. For reversible reactions both sums are equalled.

- ➢ Give initial values for all the reactants (figure C.3).

- ➢ Define the kinetics type and links between the variables, such as equilibrium constants (figure C.4)

- ➢ Define the tasks to be conducted by the program, such as time course evolution of some parameters (figure C.5).

- ➢ Specify some parameters of the data file with experimental data, and ranges for the kinetic rate constants (figure C.6).

- ➢ Choose the parameters to be plotted along the reaction course. Run the program to obtain the evolution of the chosen parameters (figure C.7).

- ➢ Two data files are generated by the program, one containing initial and calculated parameters and other containing a concentration evolution table for all the reactants.
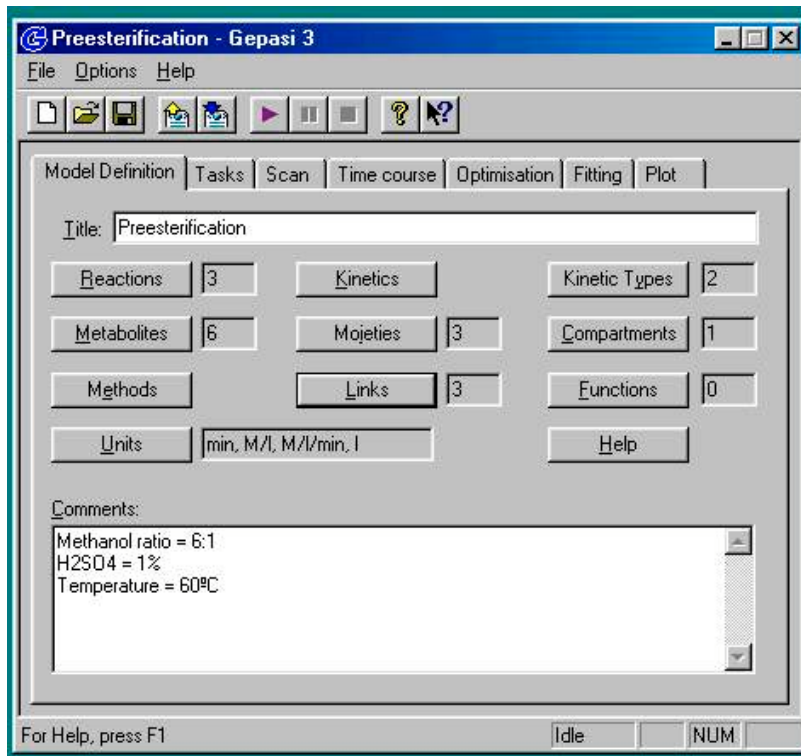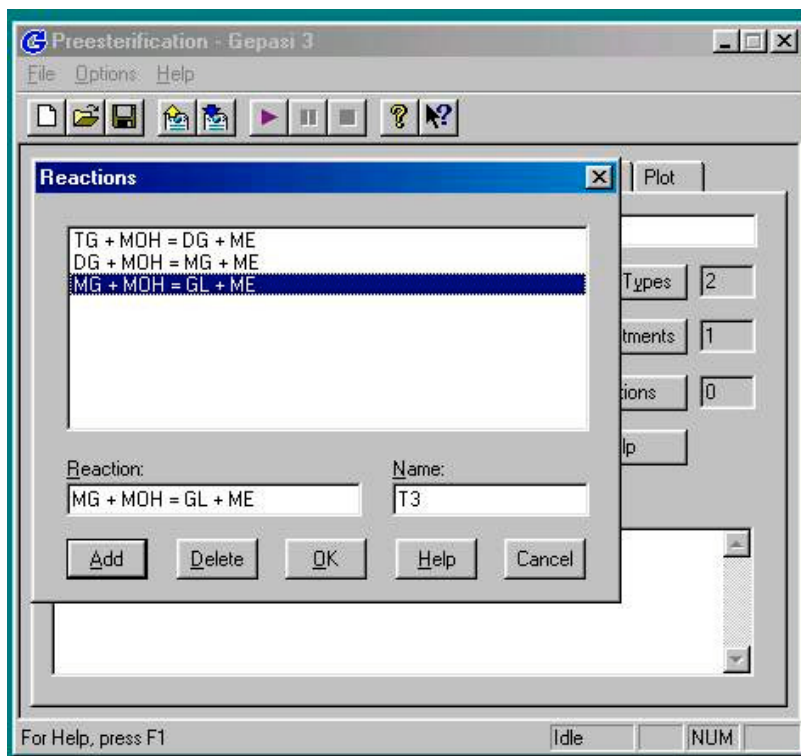
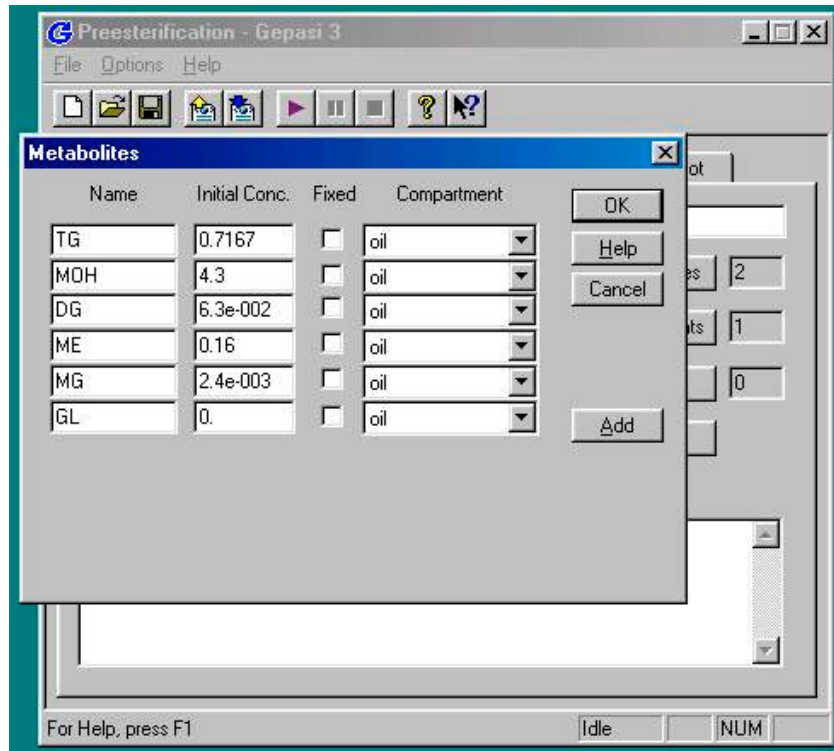Figure C.1. GEPASI's main interface

Figure C.2. Reaction mechanism

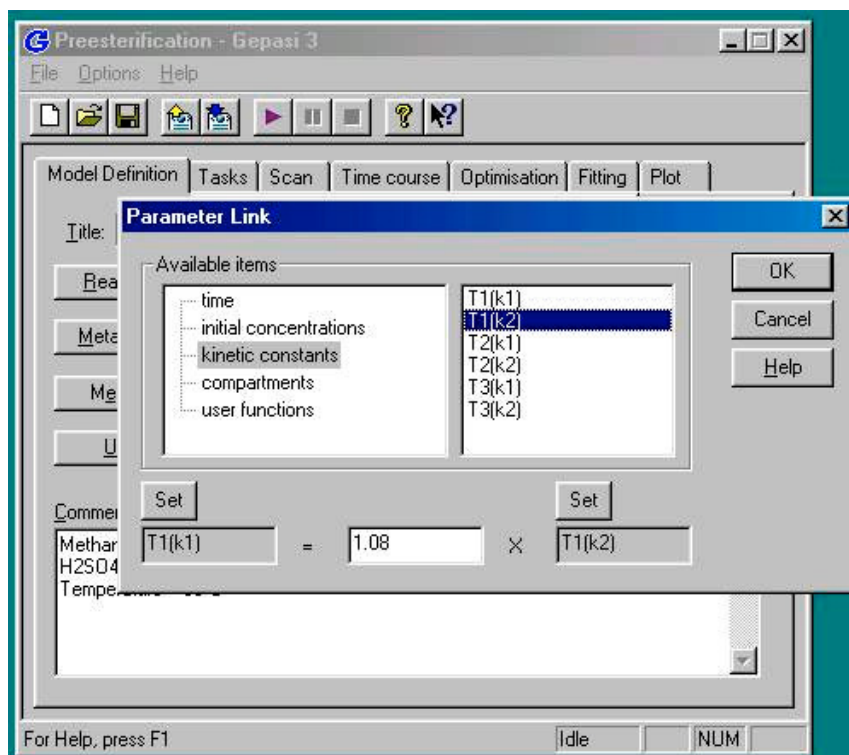Figure C.3. Initial reactants concentration

Figure C.4. Definition of equilibrium relations

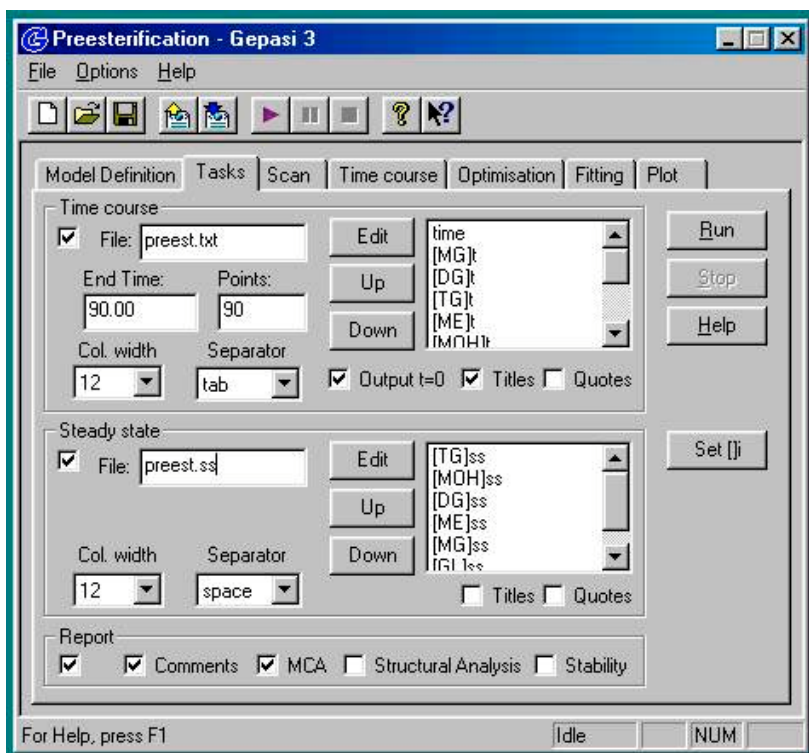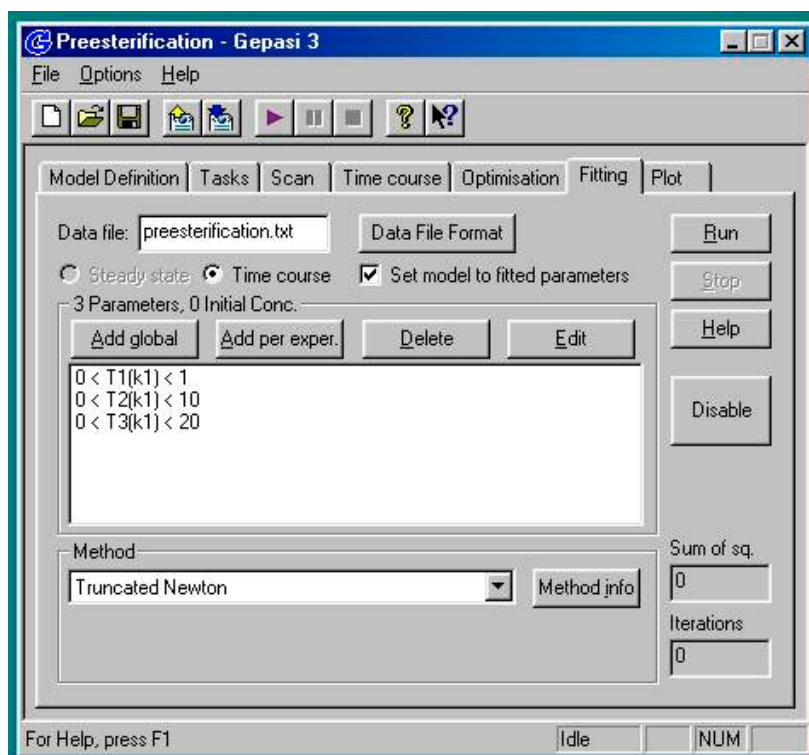Figure C.5. Simulation parameters and output file
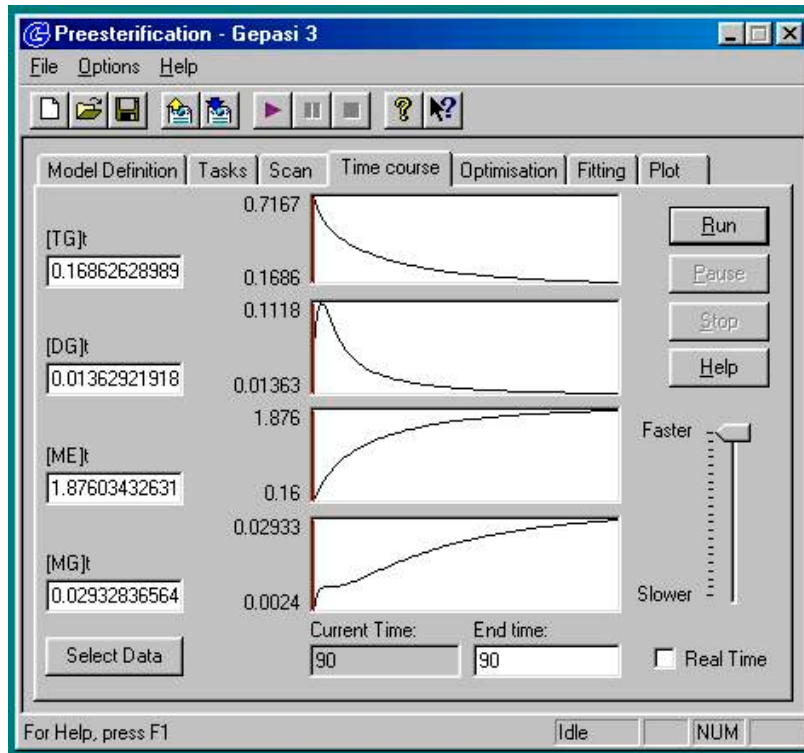

Figure C.6. Fitting parameters and data file

Figure C.7. Time curse simulation for the reactants composition