dti

LIPASE ALCOHOLYSIS OF TRIGLYCERIDES TO PRODUCE TALLODIESEL AS A TRANSPORT FUEL

CONTRACT NUMBER: B/U2/00802/00/REP

URN NUMBER: 06/1096

dti

The DTI drives our ambition of 'prosperity for all' by working to create the best environment for business success in the UK.
We help people and companies become more productive by promoting enterprise, innovation and creativity.

We champion UK business at home and abroad. We invest heavily in world-class science and technology. We protect the rights of working people and consumers. And we stand up for fair and open markets in the UK, Europe and the world.

Lipase Alcoholysis of Triglycerides to Produce Tallodiesel as a Transport Fuel (TALLODIESEL)

Contractor
BLC Leather Technology Centre

Partners
Biocatalysts Limited
Hutchings & Harding Ltd
Pittards plc Shoe and Leathergoods
Scottish Leather Group Ltd

Prepared by
BLC Leather Technology Centre

The work described in this report was carried out under contract as part of the DTI Technology Programme: New and Renewable Energy, which is managed by Future Energy Solutions. The views and judgements expressed in this report are those of the contractor and do not necessarily reflect those of the DTI or Future Energy Solutions.

© Crown copyright 2006

EXECUTIVE SUMMARY

The research carried out was designed to test the efficacy of enzyme induced tallodiesel production as a potential for the next generation of transport fuel use. The aim was primarily to test the technical innovation, then to assess the economic potential and explore opportunities for application to market within the next 25 years.

Biodiesel is an alternative to petroleum-based diesel fuel made from renewable resources such as vegetable oils or animal fats. Chemically, it comprises a mix of mono-alkyl esters of long chain fatty acids. A lipid transesterification production process is normally used to convert the base oil to the desired esters and remove free fatty acids. The biggest source of feedstock for biodiesel production is oil from crops or other similar cultivatable material. Plants utilize photosynthesis to convert solar energy into chemical energy. It is this chemical energy that biodiesel stores and is released when it is burned. Therefore plants can offer a sustainable oil source for biodiesel production.

Animal fats similarly contain chemical energy that is released when burned. However they are limited in supply and it would not be efficient to raise animals simply for their fat. Producing biodiesel with animal fat that would have otherwise been discarded i.e. from the tanning industry, could however replace a small percentage of petroleum diesel usage and provide an environmentally benign disposal route for this material. Unfortunately the chemical transesterification of tallow is restricted by the high content of free fatty acids. Chemical esterification of FFA liberates water which may cause hydrolysis and saponification of the fat feedstock, leading to the production of soaps. This will negatively affect the yield of the reaction and the recovery of the biodiesel product.

Currently there is no commercially viable process for converting such animal residues (tallow/fat) into biofuel in the UK. The UK Rendering Association estimates that over 200,000 tonnes of tallow are derived from animal carcass rendering annually. This is in comparison to some 60 million tonnes of tallow/fats globally. This material is either disposed of or sold as a low grade raw material for industrial application.

This project aimed to provide technological proof for the biochemical conversion of low-grade tallow into tallodiesel by enzyme mediated alcoholytic transesterification of fats and free fatty acids to alkyl esters. It also intended to support the potential roll out of this technology via a techno-economic study (and initial LCA) to determine an economically beneficial conversion.

In the initial stages of the research the aim was to characterise and formulate the stoichiometry of tallow and emulsified fat sources from a selection of tanning industry production facilities. Although the fats are recovered at the early stages of the leather process the production methods contain variations and the raw material sources are not necessarily common, the biggest difference being between the species of animals processed.

Tallow samples were acquired from four tanneries, three tanneries being processors of bovine material, the fourth tannery a processor of ovine material. The iodine values were as expected for saturated animal fats although the ovine tallow was very low compared to reported values. The saponification values were also similar to reported values. The unsaponifiable matter values of two tanneries were also close to reported values. However, the values for the tallow from the other two tanneries were considerably higher than reported. As expected, the free fatty acid content of all the tallows was high.

There are many potential enzyme materials that could have the potential for fat breakdown but their performance under a number of different reaction conditions is not necessarily suitable. It was therefore necessary to screen enzyme sources, determine the enzyme kinetics and optimise the efficiency of conversion of tallow, thereby determining the suitability of the enzymes as agents for tallodiesel production.

Nine enzymes were investigated for the synthesis of ethyl esters from the sample tallows. TLC analysis of the reactions suggested that most of the enzymes produced esters and that the concentration of triglyceride present (by visual assessment of the triglyceride spot on the TLC plate) was inversely proportional to the concentration of esters formed. The presence and identity of the esters was confirmed by GC-MS. No esters were detected in the treated ovine tallow, suggesting that the activity of the enzymes had been inhibited.

The detected compounds were esters of myristic acid, palmitic acid, 9-palmitic acid, stearic acid and oleic acid, the fatty acids typically associated with bovine tallow. Several of the enzymes showed very high apparent conversion efficiencies. The distribution of esters when compared to reported figures for FAME of beef tallow, suggests that the conversion was incomplete. However, it is clear from the data and the observed reduction in solids in the reaction mixtures, that significant conversion of the tallow to esters had occurred.

Of the nine enzymes tested in this work, three were seen to effectively convert the tallow into ethyl esters under the experimental conditions evaluated (> 70% conversion). In the laboratory, (10g of tallow), using an enzyme offer equivalent to £0.15 per kg of tallow, conversion efficiencies of 80% were achieved (80 % tallodiesel 8% glycerol, 12% fatty matter residue). Given the correct agitation conditions scale-up was expected to be successful.

Having characterised the tallow and identified a number of suitable enzymes the subsequent phase explored the chemical and physical properties of the produced fuel to determine its acceptability for application in conventional engines or combined heat and power plants.

Although it was anticipated that the residues would only be a minor proportion of the products of the reaction, these require consideration. Exploration of the quantity, character and opportunities for any residue produced was undertaken to identify potential disposal options

Given the already high cost of transport fuel, tallodiesel is unlikely to be considered as an option unless the costs of production are at least comparable to others fuels. Biodiesel is already favoured with an attractive taxation rate in comparison to conventional petroleum based fuels; however enzymes are traditionally expensive materials that contribute a major portion of the costs of production. An investigation of the potential costs of biodiesel production was therefore an essential aspect of the project, illustrating that a technically feasible approach could be progressed to an economic reality.

The various disposal routes for animal fleshings were investigated and the production of biodiesel was found to be of potential economic interest. Landfilling the waste incurs cost. Composting, (where possible), also results in incurred costs but these are lower than for landfill due to the avoidance of landfill tax. By contrast even a medium sized tannery may be able to produce biodiesel from tallow in a profitable manner and a larger tanneries should certainly be capable of it.

The project research indicated that enzyme mediated alcoholysis of tallow is a potentially viable route for the production of biodiesel, however efficient scale up has not been achieved. There is a good indication that the method would provide an ideal route for the disposal of animal by-product and that the added value would result in a no-cost option that has every potential for resulting in profit, provided that correct economies of scale apply.

It is apparent, however, that due to the interface conditions between the enzyme, the alcohol and the tallow that the mechanics of the reaction, i.e. mixing in the reaction vessel, is likely to be of crucial importance. The work to date indicates that at small scale at least, the chemistry works. However, simply scaling up the masses of the reactants is not enough to ensure that the reaction will proceed in bulk. In order to progress the technology to a commercially viable operation it appears that further investigation will be required, specifically with regard to scale up and mixing. This course of action may also be a possible means of reducing the amount of enzyme required and hence the cost of conversion. It is also possible that the length of time for conversion is a function of the interface conditions between the reactants. Again, investigation of the mechanics of mixing indicates potential for significant reduction in reaction times.

CONTENTS

1. REVIEW OF BIODIESEL

1.1. Background

Biodiesel is an alternative to petroleum-based diesel fuel made from renewable resources such as vegetable oils or animal fats. Chemically it comprises a mix of mono-alkyl esters of long chain fatty acids. A lipid transesterification production process is normally used to convert the base oil to the desired esters and remove free fatty acids. After this processing, biodiesel has combustion properties very similar to those of petroleum diesel, and can replace it in most current uses. However, it is at present most often used as an additive to petroleum diesel, usually in the proportions of 20% biodiesel to 80% petroleum diesel and referred to as B20.

Unlike petroleum based diesel, biodiesel is biodegradable, non-toxic and it significantly reduces toxic and other emissions when burned as a fuel. The most common form uses methanol to produce methyl esters, though ethanol can be used to produce an ethyl ester biodiesel. A by-product of the transesterification process is the production of glycerol.

Currently, biodiesel is more expensive to produce than petroleum diesel, which is often stated as the primary factor keeping it from being in more widespread usage. Favourable rates of duty to promote the use of biofuels could address this situation. Economies of scale in biodiesel production, and the rising cost of petroleum, may reduce, eliminate, or even reverse this cost differential in the future. Current worldwide production of vegetable oil and animal fat, however, is not enough to replace liquid fossil fuel use.

1.2. Source materials

A variety of bio lipids can be used to produce biodiesel. These include virgin oils, waste oils and animal fats. The virgin oil feed stocks are mainly rapeseed oil, soybean oil, mustard seed oil, palm oil, hemp and algae. The most common amongst these are rapeseed oil and soybean oil. Animal fats used for biodiesel include tallow, lard, and yellow grease .

Waste vegetable oil (WVO) is the best source of oil to produce biodiesel. However, the available supply is drastically less than the amount of petroleum-based fuel that is burned for transportation and home heating in the world. Although it is economically viable to use WVO to produce biodiesel, it is even more profitable to convert WVO into other products such as soap. Hence, most WVO that is not dumped into landfills is used for these other purposes.

The biggest source of feedstock for biodiesel production is oil from crops or other similar cultivatable material. Plants utilize photosynthesis to convert solar energy into chemical energy. It is this chemical energy that is stored by the biodiesel and is released when it is burned. Therefore, plants can offer a sustainable oil source for biodiesel production. In Europe rapeseed is the most common base oil used in biodiesel production. In India and Southeast Asia, the Jatropha tree is used as a significant fuel source, and it is also planted for watershed protection and other environmental restoration efforts. Malaysia and Indonesia are starting pilot-scale production from palm oil. Soybeans are not a very efficient crop solely for the

production of biodiesel, but their common use in the United States for food products has led to soybean biodiesel becoming the primary source for biodiesel in that country.

There is ongoing research into finding more suitable crops and improving oil yield. Using the current crops, vast amounts of land and fresh water would be needed to produce enough oil to completely replace fossil fuel usage.

Animal fats are similarly limited in supply, and it would not be efficient to raise animals simply for their fat. However, producing biodiesel with animal fat that would have otherwise been discarded, i.e. from the tanning industry, could replace a small percentage of petroleum diesel usage.

1.3. Production Methods

1.3.1. Biodiesel by Esterification Process

The traditional technology to produce biodiesel is through “transesterification”, a process that combines vegetable oils, animal fats, and/or microalgal oils with alcohol (ethanol or methanol) in the presence of a catalyst (sodium or potassium hydroxide) to form fatty esters (ethyl or methyl ester). Converting triglyceride oils to methyl or ethyl esters through a transesterification process reduces the molecular weight to one-third that of the oil, reduces the viscosity by a factor of eight, and increases the volatility.

The most important variables that influence the transesterification reaction time and conversion efficiency are temperature, catalyst type and its concentration, alcohol to ester ratio, and stirring rate. Fatty acid methyl esters are made by stirring fat or oil with methanol in the presence of a catalyst, commonly sodium or potassium hydroxide. Typical reaction conditions of 70 ^{o}C and a one-hour contact time result in 99% of the tallow being converted to esters. Crude glycerol is separated from the methyl esters by settling or centrifugation. The ester passes through a purification stage to give the final product. Glycerol is processed to recover methanol for recycling to the reaction vessel and to give pure glycerol product for sale. Purity of reactants, for example, presence of water, free fatty acids, and other contaminants found in unrefined oils (or other feedstocks) also needs consideration.

1.3.2. Biodiesel by Hydrogenation Process

Hydrogenation is a process in which biomass is mixed with conventional diesel through heating in the refinery process in such a way as to create a product chemically very similar to petroleum diesel, but somewhat more environmentally friendly.

CANMET Energy Technology Centre (CETC), have developed a process that converts vegetable oils, waste greases, animal tallow and other feedstocks containing triglycerides and fatty acids into a high-cetane, low-sulphur diesel fuel blending stock called SuperCetane. The process employs a conventional commercial refinery hydrotreating catalyst and hydrogen. The product generated is a hydrocarbon liquid, which can be distilled into 3 fractions: naphtha, middle distillate

and waxy residues. The middle distillate, which makes up most of the product, is the SuperCetane. It has a cetane number of around 100—comparable to commercial cetane additives. The specific gravity of SuperCetane is similar to regular diesel while its viscosity is similar to biodiesel. It is 97% biodegradable as compared to 45% for regular diesel.

1.3.3. Biodiesel by enzyme mediated transesterification

Currently, there is no commercially viable process converting such animal residues (tallow / fat) into biofuel in the UK. The UK Rendering Association estimates that over 200,000 tonnes of tallow are derived from animal carcass rendering annually. This is in comparison to some 60 million tonnes of tallow/fats globally. This material is either disposed of or sold as a low grade raw material for industrial application.

Conversion of this waste substrate to a biofuel (tallodiesel) could contribute a small proportion to the current UK diesel consumption and has potential worldwide commercial applicability. Other applications for fuels derived in this manner could include replacement of domestic heating oil and application in stationary combined heat and power (CHP) generation. It also contributes to a solution for a UK and EU disposal problem of animal by-products and the fatty products of surfactant based aqueous degreasing of hides & skins. It also provides, through utilisation of an existing resource, an alternative to the associated costs of 'energy crop' production. However, chemical transesterification of tallow is restricted by the high content of free fatty acids. Chemical esterification of FFA liberates water, which may cause hydrolysis and saponification of the fat feedstock, leading to the production of soaps. This will negatively affect the yield of the reaction and the recovery of the biodiesel product.

Enzymes are amongst the most important biocatalysts carrying out novel reactions in both aqueous and non-aqueous media. This is primarily due to their ability to utilise a wide spectrum of substrates, high stability towards extremes of temperature, pH and organic solvents, and chemo-, regio- and enantioselectivity. The enantioselective and regioselective nature of lipases have been utilised for the resolution of chiral drugs, fat modification, synthesis of cocoa butter substituents, biofuels, and for synthesis of personal care products and flavour enhancers (Gerhartz, 1990; Priest, 1992).

Lipases (triacylglycerol acylhydrolases) belong to the class of serine hydrolases and therefore do not require any cofactors. The natural substrates of lipases are triacylglycerols, having very low solubility in water. Under natural conditions, they catalyse the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. Under certain experimental conditions, such as in the absence of water, they are capable of reversing the reaction. The reverse reaction leads to esterification and formation of glycerides from fatty acids and glycerol. They will also catalyse the transesterification with alcohol, of fats, oils and FFA, producing alkyl esters (biodiesel).

Figure 1 illustrates lipase mediated alcoholytic transesterification of fats and free fatty acids to alkyl esters (biodiesel).

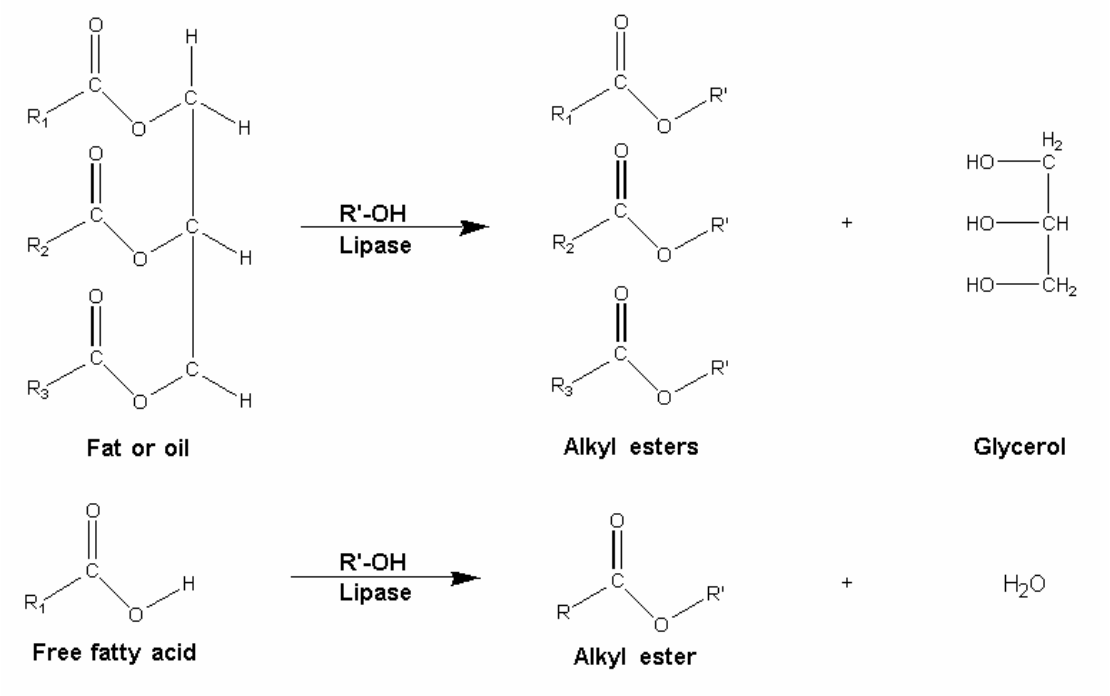

Figure 1 Lipase mediated alcoholytic transesterification of fats and free fatty acids to alkyl esters

The use of lipases to catalyse the transesterification of fatty acids to alkyl esters for use as biodiesel, has not yet been commercially applied. However, the process has been investigated experimentally. The reaction involves lipase mediated transesterification of fats or FFA with alcohol producing alkyl esters and glycerol or water (Figure 1).

2. INFLUENCE OF FEEDSTOCK CHEMICALS

Biodiesel is chemically simple, since no more than six or seven fatty acid esters make up the biodiesel mixture. Different esters vary a great deal in terms of important fuel properties, such as: Cetane Number (CN); density, viscosity, melting point, cold flow characteristics (such as Cloud and Pour points), heating value, and degree of saturation. Different vegetable oils and animal fats may contain different types of fatty acids. The fuel-related biodiesel properties will be dependant upon the range of esters produced which will in turn be affected by the choice of raw material (Table 1). The data for actual biodiesel fuels, methyl and ethyl esters of various vegetable oils and tallow has been determined (Graboski, 1997), and the results do indeed indicate small differences attributable to the use of different raw materials.

Table 1 Fuel properties of Soy bean esters (from Schwab 1987)

Ester	Viscosity(mm^2/s)	Cetane No	Heat of Combustion (MJ/kg)	Cloud Point (^oC)	PourPoint (^oC)
Soy methyl ester	4.08	46.2	39.8	2	-1
Soy ethyl ester	4.41	48.2	40.0	1	-4
Soy butyl ester	5.24	51.7	50.7	-3	-7

The chemical composition and properties of biodiesel also depends on the length and degree of unsaturation of the fatty acid alkyl chains. Fatty acids can be saturated or unsaturated. A saturated acid is one that cannot chemically add hydrogen, whereas an unsaturated acid can be hydrogenated. The saturated acids exhibit higher freezing points than the unsaturated acids. The boiling points of the acids are dependent on the length of the carbon chain but are nearly independent of the degree of unsaturation. The effects of chemical structure on melting and boiling points also apply to esters of the fatty acids. In view of significant variation in biodiesel properties based on the raw material, characterisation of the tallow from UK tanneries was undertaken to determine the variability of this feedstock in particular.

2.1. Tallow characterisation tests

Tallow was acquired from four tanneries (“W”), (“X”), (“Y”), and (“Z”). The first three tanneries are processors of bovine material, the fourth tannery (“Z”) is a processor of ovine material. These were subject to a number of chemical tests (described below) and the results given in Table 2.

IODINE VALUE

The degree of saturation of the tallow samples was assessed by determination of the iodine value.

SAPONIFICATION VALUE
The saponification value (SV) is equal to the number of milligrams of KOH required to saponify 1g of the tallow.
The SV was used to determine the saponification equivalents by:

$$SE = m/SV$$

where SV = and m = molecular weight of KOH

The value of SE was then converted to an approximate mean molecular weight of triglycerol according to the following:

$$Mtag = SE \times 3$$

UNSAPONIFIABLE MATTER
Unsaponifiable matter was determined by adding tallow to ethanolic KOH and refluxing. After a washing regime the extract was dissolved in ethanol and titrated against ethanolic KOH with phenolphthalein indicator to determine the mass of residual material, which is expressed as a % of the original sample mass.

TOTAL FREE FATTY ACIDS
The total free fatty acid content of the tallows was determined by titration. The total free fatty acid is expressed as % oleic acid by the following equation:

$$\%FFA = 2.82V/M_0$$

where V = volume of KOH in ml and M_0 = mass of the original sample.

2.2. Results

The iodine values are as expected for saturated animal fats although the figure for ("Z") is very low compared to reported values. The calculated saponification values are also similar to reported values. The unsaponifiable matter values of the ("X") and ("Y") samples are close to reported values. However, the values for the tallow from ("W") and ("Z") are considerably higher than reported, possibly indicating contamination of the samples. As expected, the free fatty acid content of all the tallows was high.

Table 2 Chemical analysis of industrial tallow samples

Tallow source	Iodine Value	Saponification Value	Unsaponifiable material (% m/m)	Total free fatty acids (%)
("W")	45.7	250.9	3.0	6.6
("X")	46.1	133.0	1.1	2.1
("Y")	35.1	162.6	1.8	2.8
("Z")	2.6	202.6	3.8	2.3

2.3. Discussion

The chemical characteristics of the tallow from ("Y") and ("X") were similar to that reported for animal tallow (Rossell, 1986). The iodine values, an indicator of the degree of saturation of the fats, were low (35.1 and 46.1, for ("Y") and ("X"), respectively). This is typical of animal fats, which are mostly saturated. Tallow from ("Z") had a very low iodine value, suggesting very little unsaturation. A lack of unsaturated bonds would not affect the synthesis of esters from the fat. However, the degree of saturation may affect the performance characteristics of any fuel synthesised from those tallows. Highly saturated esters are reported to have higher cetane numbers and greater stability during storage but poorer cold flow characteristics than unsaturated esters.

The saponification values for all the tallows, and unsaponifiable matter values for the ("Y") and ("X") tallow were also in the range of reported values. However, the unsaponifiable matter content of ("Z") tallow was considerably higher, suggesting that the material may have been contaminated. This may have occurred during the processes used for the recovery of the tallow. While the presence of unsaponifiable matter is reported not to affect the performance of biodiesels, the material present may explain the failure of any of the tested lipases to produce esters from the ("Z") tallow. To determine this conclusively however would require further evaluation. As expected, the free fatty acid content of the tallow was significant (> 2%). Free fatty acid contents of 0.6% have been shown to cause significant inhibition of the chemical esterification of tallow. As such, the tallow described here would be entirely unsuitable as a substrate for biodiesel synthesis via chemical esterification.

2.4. Enzyme assessment

The initial esterification experiments were carried out by heating tallow in a water bath until melted. 10g was added to a 150 ml Erlenmeyer flask. The flask was closed to prevent evaporation of ethanol. Lipase was added to the tallow and mixed until homogenous. The flask was placed into an orbital shaker at 40 ^{o}C and shaken at 200 rpm. Ethanol was added into the flask at 100 µl per hour for 3 hours and subsequently at 200 µl per hour, until a total volume of 2.5 ml of ethanol had been added. Control samples, without ethanol, were also shaken for 48 h. After 48 h of incubation, the flask was removed from the shaker and the contents analysed.

THIN LAYER CHROMATOGRAPHY

Tallow and lipase-treated samples were analysed for the presence of mono-, di- and triglycerides, fatty acids and fatty acid esters, by thin layer chromatography (TLC). Samples were dissolved in hexane:diethyl ether (1:1, v/v) to a concentration of 100 mg ml^{-1}. Analtech Silica G TLC plates were cleaned in the TLC solvent, oven dried at 100°C and cooled. A 1µl aliquot of sample was applied 15mm from the base of the plate and the plate run in hexane:diethyl ether: acetic acid (70:30:1 or 80:20:2, v/v) until the solvent front was 10mm from the top of the plate. The plate was then developed by spraying with 50% sulphuric acid and charring at 100°C for 3 mins.

ESTERIFICATION OF FATTY ACIDS TO FATTY ACID METHYL ESTERS (FAME)

Prior to analysis by GC-MS, the samples were derivitised to fatty acid by refluxing 0.1g (± 0.001g) of lipid with 4 ml of 0.1M methanolic NaOH for 10 min. Five millilitres of boron trifluoride methanol complex were added and refluxed for 2 min. Five millilitres of heptane were added and the sample cooled. The flask was then filled to the neck with saturated sodium chloride solution and the organic layer pipetted into glass vials.

GC-MS ANALYSIS OF FAME
Derivitised and lipase-treated samples were analysed for FAME and fatty acid ethyl esters, respectively, by GC-MS. Analysis was carried out with a Varian CP-3800 GC and a Varian Saturn 2000 MS (EI mode, 40 – 650 m/z) using a Varian CP-SIL5 60m column (0.25mm i.d. x 0.1mm film thickness). Chromatography conditions were as follows: helium carrier gas; injector temperature 275°C with a 50% split ratio; 10µl injection; oven temperature, 50°C for 2 min then 10°C min^{-1} to 310°C, then held for 5 min; total run time 33 min.

The sample identities and test conditions are shown in Table 3.

2.5. Results

The results of the TLC analysis of the ester products of lipase treatment of ("Y") and ("X") tallow are shown in Figure 2 and 3, respectively. As can be seen, no esters were observed in the control treatments (tallow alone, without enzyme or without ethanol). Ester products were observed in samples D, H, L, N, R and T, following treatment of the ("Y") tallow. Additionally, the spot associated with triglycerides was reduced (subjective visual inspection), compared to the controls in sample H, and significantly reduced in samples L and N. This suggests that the formation of esters was due to the action of the lipase on the triglycerides. Similarly, esters were detected in samples D, F, H, J, L, N, P, R and T of lipase-treated or control samples of ("X") tallow. Significant reductions in triglycerides were observed in samples H, L & N. The results indicated that, as a result of enzyme treatment of both tallows, esters had been produced. The TLC also showed triglyceride was the significant component of both tallows. No esters were detected following lipase treatment of ("Z")s' tallow.

The presence of esters and their identities was confirmed by GC-MS analysis. Esters were detected in all of the samples of ("Y") and ("X") tallow treated with lipase and ethanol. The esters detected were ethyl esters of myristic acid (C14), palmitic acid (C16), 9-palmitic acid (C16), stearic acid (C18) and oleic acid (C18). These esters were also detected after esterification (FAME) of ("W")s tallow (data not shown). The concentrations and relative abundance of each ester was calculated from the relative area of the methyl ester standards used for the identification of the ester products (Table 3 & 4). Relatively high conversion rates of the ("Y") tallow were calculated with the highest conversions measured in Sample L (50.6%) and N (79.9%). Similarly high values were observed in all the treated ("X") samples, with the exception of sample T. However, the apparent conversion efficiency (mass of sample / mass of tallow) in samples H, L & N were greater than 100%, suggesting a bias towards the esters when sampling for the GC-MS analysis. No esters were detected in the samples of ("Z")s tallow treated with lipases.

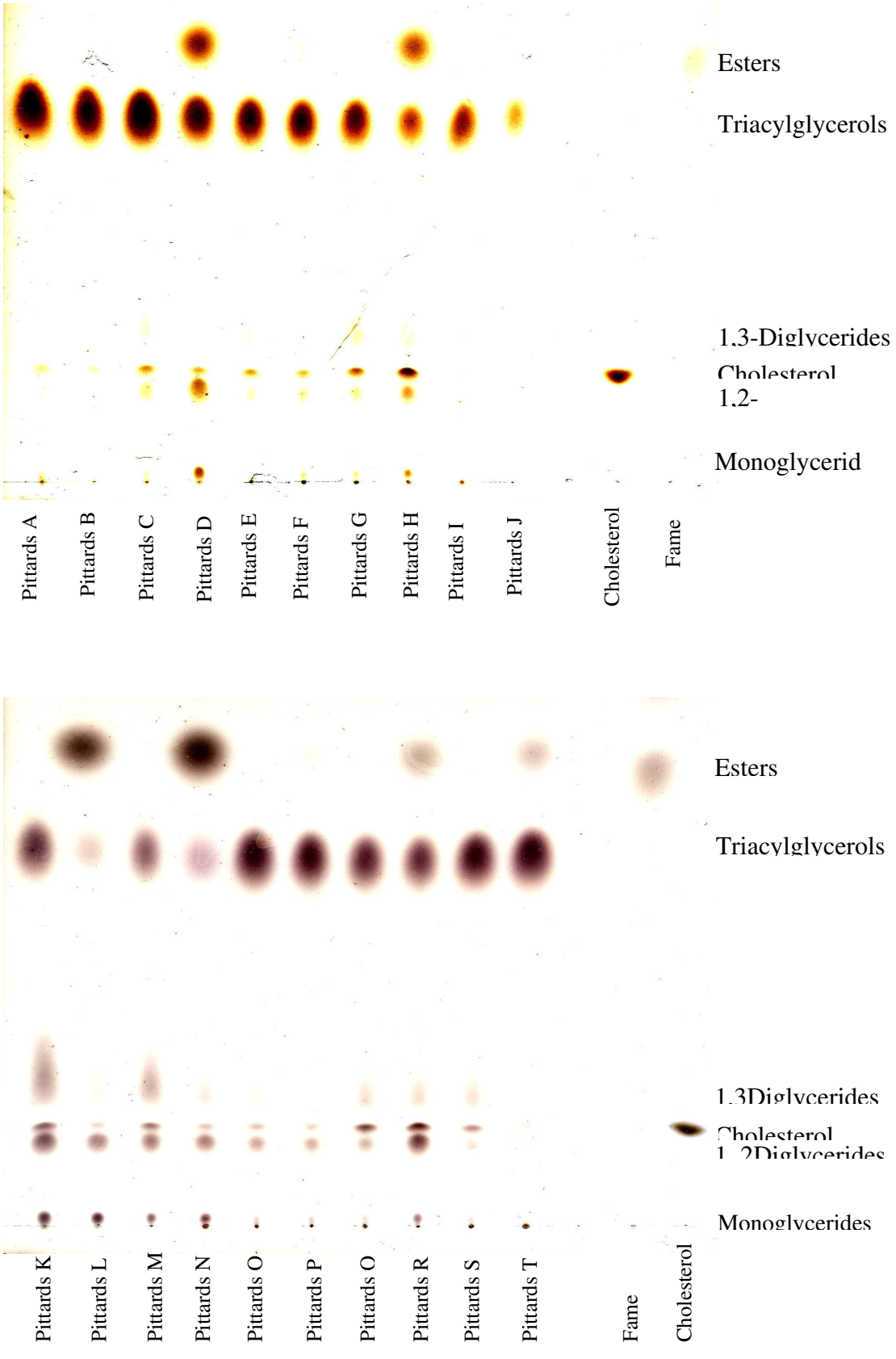

Figure 2 TLC analysis of tallow ester following lipase treatment of ("Y") tallow.

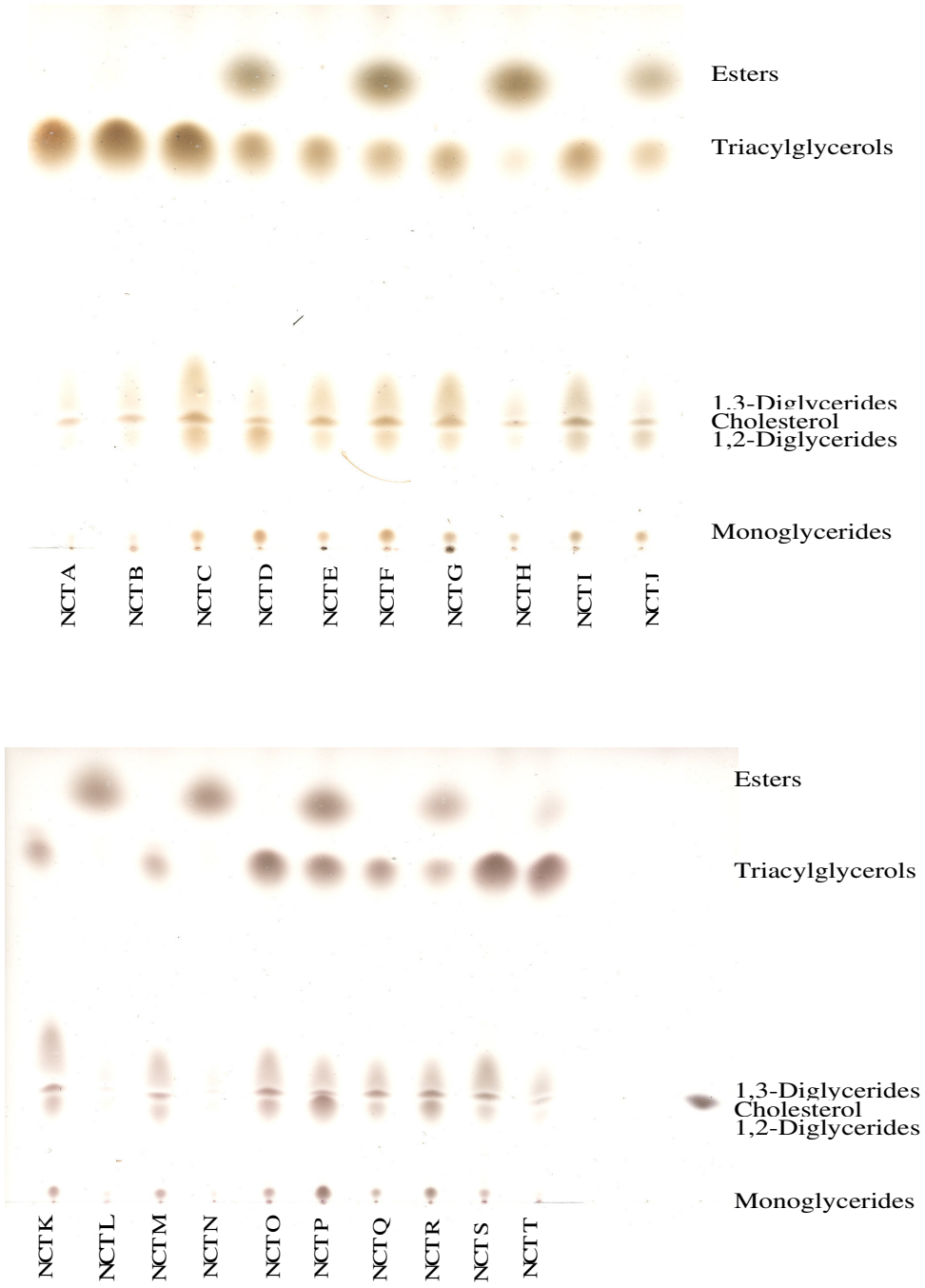

Figure 3 TLC analysis of tallow ester following lipase treatment of ("X") tallow.

Table 3 Sample identities and conditions of tallow samples treated with various lipases

Sample	Tallow (g)	Ethanol	Lipase (g)
Control A	10 g	-	-
Control B	10 g	2.5 ml	-
Control C	10 g	-	0.5 g Enzyme “A”
Sample D	10 g	2.5 ml	0.5 g Enzyme “A”
Control E	10 g	-	0.5 g Enzyme “B”
Sample F	10 g	2.5 ml	0.5 g Enzyme “B”
Control G	10 g	-	0.5 g Enzyme “C”
Sample H	10 g	2.5 ml	0.5 g Enzyme “C”
Control I	10 g	-	0.5 g Enzyme “D”
Sample J	10 g	2.5 ml	0.5 g Enzyme “D”
Control K	10 g	-	0.5 g Enzyme “E”
Sample L	10 g	2.5 ml	0.5 g Enzyme “E”
Control M	10 g	-	0.5 g Enzyme “F”
Sample N	10 g	2.5 ml	0.5 g Enzyme “F”
Control O	10 g	-	0.5 g Enzyme “G”
Sample P	10 g	2.5 ml	0.5 g Enzyme “G”
Control Q	10 g	-	0.5 g Enzyme “H”
Sample R	10 g	2.5 ml	0.5 g Enzyme “H”
Control S	10 g	-	0.5 g Enzyme “I”
Sample T	10 g	2.5 ml	0.5 g Enzyme “I”

Table 4 Quantification and distribution of ethyl esters detected by GC-MS following lipase treatment of (“Y”) tallow. Values calculated from methyl ester standards

	Mass (g) / % distribution of ethyl esters detected in lipase treated samples					
Sample	Ethyl myristate	Ethyl 9-palmitate	Ethyl palmitate	Ethyl oleate	Ethyl stearate	Total mass (g)
D	0.15 / 5.49	0.24 / 8.66	1.42 / 52.17	0.45 / 16.79	0.46 / 16.89	2.72
F	0.02 / 3.08	0.11 / 13.52	0.46 / 58.46	0.13 / 16.67	0.07 / 8.27	0.79
H	0.20 / 6.35	0.38 / 11.71	1.52 / 47.04	0.73 / 22.72	0.39 / 12.18	3.22
J	0.06 / 4.07	0.12 / 8.72	0.78 / 59.55	0.19 / 15.09	0.17 / 12.57	1.31
L	0.35 / 6.98	0.61 / 12.10	2.79 / 54.95	1.15 / 22.70	0.16 / 3.27	5.06
N	0.48 / 6.01	0.90 / 11.30	3.64 / 45.46	1.56 / 19.57	1.41 / 17.66	7.99
P	0.01 / 4.55	0.06 / 15.11	0.13 / 38.76	0.05 / 15.83	0.08 / 25.75	0.33
R	0.14 / 5.52	0.21 / 7.76	1.55 / 59.64	0.32 / 12.32	0.38 / 14.76	2.60
T	0.04 / 3.21	0.10 / 9.33	0.60 / 56.66	0.20 / 19.38	0.12 / 11.42	1.06

Table 5 Quantification and distribution of ethyl esters detected by GC-MS following lipase treatment of ("X") tallow. Values calculated from methyl ester standards

	Mass (g) / % distribution of ethyl esters detected in lipase treated samples					
Sample	**Ethyl myristate**	**Ethyl 9-palmitate**	**Ethyl palmitate**	**Ethyl oleate**	**Ethyl stearate**	**Total mass (g)**
D	0.45 / 5.83	0.89 / 11.72	3.92 / 51.21	1.25 / 16.24	1.14 / 15.00	7.65
F	0.32 / 5.73	0.85 / 15.31	2.43 / 44.06	1.28 / 23.13	0.65 / 11.77	5.53
H	0.55 / 4.81	1.27 / 11.01	3.58 / 31.27	2.21 / 19.30	3.85 / 33.61	11.46
J	0.29 / 5.88	0.59 / 11.69	2.22 / 43.93	1.12 / 22.12	0.83 / 16.38	5.05
L	0.78 / 4.64	1.55 / 9.10	4.69 / 27.65	2.87 / 16.95	7.06 / 41.66	16.95
N	0.75 / 4.67	1.48 / 9.13	4.92 / 30.26	2.85 / 17.59	6.23 / 38.35	16.23
P	0.22 / 4.04	0.67 / 12.74	1.90 / 36.29	0.93 / 17.82	1.52 / 29.11	5.24
R	0.27 / 5.52	0.83 / 17.78	1.81 / 38.47	0.89 / 18.89	0.91 / 19.34	4.71
T	0.05 / 4.97	0.25 / 24.48	0.34 / 33.43	0.24 / 23.84	0.13 / 13.28	1.01

2.6. Discussion.

TLC analysis of the reactions suggested that most of the lipases produced esters from both the ("Y") and ("X") tallow and that the concentration of triglyceride present (visual assessment of the triglyceride spot on the TLC plate) was inversely proportional to the concentration of esters formed.

No ester products were observed following analysis of the ("Z") tallow. The presence and identity of the esters was confirmed by GC-MS. Ester products from the ("Y") and ("X") tallows were measured for all nine lipases. No esters were detected in the treated ("Z") tallow, suggesting that the activity of the lipases had been inhibited. The detected compounds were esters of myristic acid, palmitic acid, 9-palmitic acid, stearic acid and oleic acid, the fatty acids typically associated with bovine tallow. Several of the lipases showed very high apparent conversion efficiencies, including conversion efficiencies of over 160% when ("X") tallow was treated with Enzyme "E" (sample L) or Enzyme "F" (sample N).

This suggests a sampling bias for the esters and may have been due to the settling of any residual tallow solids prior to sampling for GC-MS analysis, i.e. the sample was not homogeneous. The distribution of esters, when compared to reported

figures for FAME of beef tallow, also suggests that the conversion was incomplete. However, it is clear from the data and the observed reduction in solids in the reaction mixtures, that significant conversion of the tallow to esters had occurred.

Of the nine lipases tested in this work, three were seen to effectively convert the tallow into ethyl esters under the experimental conditions outlined above (> 70 % conversion).

2.7. Tallow suitability

The characteristics of the tallows analysed are typical of animal tallow, although the tallow from ("Z") had a high content of unsaponifiable material, which may have been due to a process contaminant. All three tallows contained free fatty acids at concentrations that would prohibit their use in a chemical esterification process, without pre-treatment. All nine of the screened enzymes produced esters from the ("Y") and ("X") tallow, demonstrating the applicability of lipases for the synthesis of fatty acid ester (biodiesel) from tallow.

3. PROTOCOL FOR SYNTHESIS

3.1. Protocol Development

The two most promising lipases from the lipase assessment work were investigated in more detail to optimise the levels of enzyme and the time required for conversion of the tallow into biodiesel.

The offers of enzyme used were based around commercially viable levels that could be used to make the process cost effective. The maximum offer of enzyme would cost 30p based on converting 1 litre of tallow into biofuel, which combined with the duty on diesel would bring the cost to approximately fuel pump prices.

3.2. Preliminary Protocol (laboratory scale processing)

The preliminary protocol for the production of biodiesel from tallow using lipase mediated alcoholysis was developed as a laboratory scale process.

The reaction was undertaken in two types of vessel – baffled and unbaffled (enclosed reaction vessel and open reaction vessels). The reaction was also investigated by staged additions of enzyme and in baffled and unbaffled flasks

Diesel produced based around the protocol shown was tested for a range of properties to ascertain the quality of the biofuel synthesised.

3.3. Analysis

The results for the analysis carried out on the initial scaled up tallodiesel produced are shown in Table 6.

Table 6 Testing of tallodiesel (laboratory scale process)

Test	Method	Units	Specification		Results
			Minimum	Maximum	
Density @ 15°C	EN ISO 12185	kg/m^3	860	900	870.6
Viscosity @ 40°C	EN ISO 3104	mm^2/s	3.5	5.0	5.54
Flash Point	EN ISO 3675	°C	120		44
Sulphur Content	EN ISO 20865	mg/kg		10	73
Carbon Residue	IP 13	%m/m		0.3	0.41
Cetane Number	EN ISO 5165		51		50.2
Total Contamination	EN 12662	mg/kg		24	13
Acid Value	EN 14104	mg KOH/g		5	10
CFPP	BS EN 116	°C			5

The results for the tallodiesel were very promising, although some aspects of the product needed addressing. The flash point of the product was very low which suggests that the tallow did not fully convert and there was some residual alcohol left in the fuel.

Sulphur content was (as expected) high as the product was produced from limed fleshings which would have been exposed to sodium sulphide. Also tallow is known to have a high sulphur content which is one of the problems when using it to produce biofuels.

The cetane number was lower than expected, and again this was probably due to incomplete conversion of the tallow into diesel.

Under the protocol employed full conversion of fatty acids to tallodiesel did not occur. There was a degree of settling out between the produced tallodiesel and unconverted fatty matter. The samples were therefore centrifuged to separate the solid and liquid fractions of the products. The proportions for each sample are given in Table 7 and represented graphically in Figure 4. Given that very good conversion efficiencies had been achieved in the initial experiments using 10 g of tallow sample, and the same protocol was used, this minor scale-up appears to be the cause of reduced performance. This is further indicated by the increased yield obtained in the baffled flasks compared to the unbaffled flasks. Although increased offers of enzyme would be expected to overcome this issue the reduced conversion efficiencies appear to be more closely related to the degree of agitation rather than enzyme offer.

Table 7 Proportions of tallodiesel and fatty residue after centrifugation

sample	Reaction vessel	%fat	% diesel
1	Baffled flask	58.75	41.41
2	Unbaffled flask	64.86	35.25
3	Baffled flask	49.79	50.31
4	Unbaffled flask	56.45	43.60
5	Baffled flask	80.07	19.95
6	Unbaffled flask	78.08	21.89

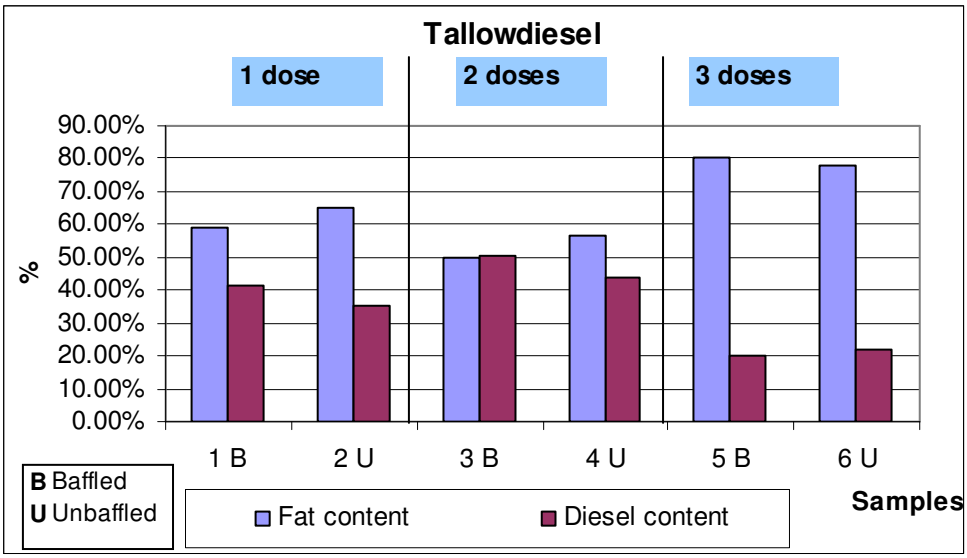

Figure 4 Proportions of tallodiesel and fatty residue after centrifugation

As can be seen in Figure 4 and Table 7 the application conditions of the lipase materially affect the amount of diesel product obtained. The results indicate that staged additions are more effective, but with the enzyme offer applied, maximum conversion efficiency appears to have been obtained with the two dose application procedure. There appears to be a slight improvement in conversion efficiency by using baffled flasks as opposed to the unbaffled flasks. The maximum conversion efficiency obtained was 50% using two applications of lipase in baffled flasks, which is lower than that previously obtained when processing smaller samples. Reduced agitation in this series of larger scale experiments results in reduced yield.

Analysis of the diesel was undertaken to determine the proportions of selected alkyl esters. The result of this analysis is given in Table 8.

Table 8 Proportions of selected alkyl ester in tallow diesel samples

	1 dose		2 dose		3 dose	
sample	**1B**	**2U**	**3B**	**4U**	**5B**	**6U**
tetradecanoic ac, ethyl ester	18.3	1.8	27.2		29.4	
ethyl 9-hexadecenoate	61.9	34.0	73.9		13.5	
hecadecanoic ac, ethyl ester	465.8	276.2	557.7		217.8	
ethyl oleate	163.9	105.1	235.3	262.7	54.8	2383.5
octodenacoic ac, ethyl ester	75.5	55.6	132.4	154.4	54.5	1797.3
total mass ppm	785.4	472.6	1026.6	417.1	370.1	4180.8
ppm sample	1120.0	1056.0	1145.0	1010.0	1065.0	1135.0
%	**70.1**	**44.8**	**89.7**	**41.3**	**34.8**	**368 ***

*This result appears to be subject to experimental error.

Further experimentation on the effects of scale confirms that agitation is a key parameter in the enzyme-mediated conversion of tallow to tallodiesel. The reaction is dependant upon the enzyme interacting with the reactants. Alterations in the effectiveness of interactions between the polar and non polar phases, (i.e. the effectiveness of the mixing action), will affect enzyme /substrate interaction and consequently the yield obtained.

When maintaining the reaction vessel and mixing hardware, but changing the mass of material reacted, the fluid flow mechanics within the vessel will change. Generally, when using an impeller in a circular reaction vessel, the mixing action decreases with increasing volume of reactants. It appears that for the reaction being investigated there is also a mass below which the mixing action is reduced (Table 9).

Table 9 Tallodiesel conversions under different mechanical action conditions

	Sample A	**Sample B**
Mass of tallow	150 g	100 g
Flask size	500 ml	500 ml
Tallodiesel (w/w conversion)	80.8 %	40.8 %
Glycerol (w/w conversion)	8.7 %	2.1 %
Unconverted fatty matter w/w conversion	10.5 %	57.1 %

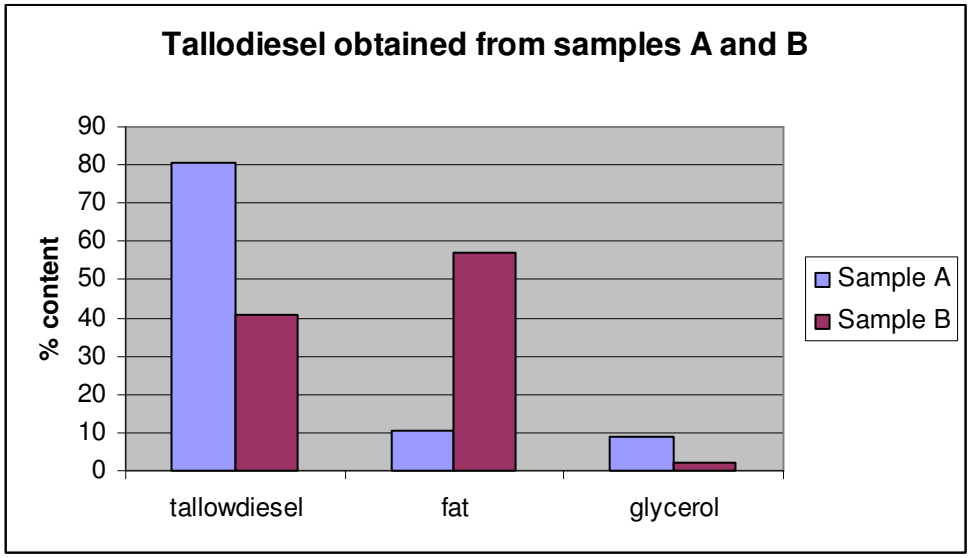

Figure 5 Tallodiesel conversions under different mechanical action conditions

Following these trials attempts were undertaken to reduce the amount of enzyme still further. In small scale trials (10g of tallow), using an enzyme offer equivalent to £0.15 per kg of tallow, conversion efficiencies of 80% have been achieved (80 % tallodiesel 8% glycerol, 12% fatty matter residue). Given the correct agitation conditions scale-up would be expected to be successful.

3.4. Scale-up

The process was repeated at a larger scale using 100 kg of mechanically recovered tallow. The protocol for the reaction was maintained although methanol was substituted for ethanol. The analysis of the resultant diesel is given in Table 10.

Table 10 Biodiesel fuel specification and lab scale results

Test	Method	Units	Specification		Results
			Minimum	Maximum	
Density @ 15°C	EN ISO 12185	kg/m^3	860	900	901.8
Viscosity @ 40°C	EN ISO 3104	mm^2/s	3.5	5.0	16.02
Flash Point	EN ISO 3675	°C	120		
Sulphur Content	EN ISO 20865	mg/kg		10	282
Carbon Residue	IP 13	%m/m		0.3	0.2
Cetane Number	EN ISO 5165		51		
Total Contamination	EN 12662	mg/kg		24	
Acid Value	EN 14104	mg KOH/g		5	22.07
CFPP	BS EN 116	°C			

The initial scale–up results indicated a conversion efficiency in the order of 60% (an accurate figure cannot be given due to local technical difficulties in separation of the products). These initial scale-up results are disappointing in comparison to the laboratory scale trials that returned a conversion efficiency of up to 80%.

It was noted in the initial laboratory scale assessments that the degree of mechanical action appeared to be a determinant factor in the efficiency of conversion. In the bulk scale trial an agitation regime of 600 rpm in the reaction vessel was specified, the same as at the laboratory scale. It is possible that the actual degree of agitation was not as effective due to the larger scale of the trial which accounts for the poorer result, suggesting that this is an area that requires further investigation. The scale-up trials were undertaken in an unbaffled reaction vessel as access to a baffled vessel was not possible. Although the laboratory scale trials indicated a minor efficiency improvement in baffled vessels this alone would not account for the reduced result obtained. It is however a further factor requiring investigation.

The analysis of the resultant diesel recorded a very high sulphur content. Given that the tallow used was mechanically recovered tallow the cause of the high sulphur content is inexplicable other than being due to prior contamination of the tallow or subsequent contamination of the diesel sample.

The importance of mechanical mixing action in the reaction has been reinforced by subsequent discussions with fluid flow mechanical engineers. In this reaction there are two immiscible phases (fat/oil and enzyme in water medium), which limits contact between the reactants. Furthermore it is necessary that the reaction products are in contact with the active site on the enzyme. The high agitation achieved in laboratory scale is not replicated simply by scaling up the volumes of reactants in a larger vessel. The mixing action must be adapted to ensure a high inter-phase boundary area and contact time with the enzyme. It is the opinion of the fluid flow mechanical engineering specialists that mixing technologies will be a critical factor in effective scale up of the process, a view supported by the reduced efficiency of conversion encountered in the scale-up investigations. The optimum mixing conditions could be readily ascertained by specialist fluid flow mechanical engineers. Certain specialist engineering companies are aware of this project and have shown interested in pursuing this line of investigation (but this would be on a commercial basis).

4. RESIDUES

The products obtained from the lipase mediated tallodiesel production process are biodiesel, glycerol and fatty acids. There can be problems with the relatively high free fatty acid content in waste oils, which make it more difficult to properly separate the glycerol and esters obtained from the transesterification process. Therefore, in selecting a feedstock, the cost of raw materials, as well as the processing cost and its effect on the quality of biodiesel and other by-products, all need careful assessment.

The best laboratory scale trials indicated a conversion efficiency of about 80%. The resultant products can be readily separated into three phases comprising biodiesel, glycerol and the remaining material that has the appearance of unconverted fatty matter.

Analysis of this residue indicates that it has very similar properties to tallow (Table 11)

Table 11 Tallow and residue analysis

	Tallow	Residual
Ash (%)	<1	<1
Grease (%)	97	99
Saponification value	140.8	151.7
Water content (%)	2.99	2.36
Glycerol (mg/L)	<0.1	0.23
Methanol content (ppm)	-	<0.5

This analysis suggests that apart from some contamination as a result of the reaction (residual methanol content, residual glycerol content) the residual fatty matter is unconverted tallow that remains in that condition either as a result of the fat composition of this fraction or because there is still scope for process efficiency improvements.

4.1. Glycerol

Glycerol is present in the form of its esters (glycerides) in all animal and vegetable fats and oils. It is obtained commercially as a by-product when fats and oils are hydrolysed to yield fatty acids or their metal salts (soaps). Glycerol is also synthesised on a commercial scale from propylene (obtained by cracking petroleum), since supplies of natural glycerol are inadequate.

Glycerol can be obtained as the by-product of the transesterification process. There are several applications of glycerol. These include:

- Medical and pharmaceutical preparations, mainly as a means of improving smoothness, providing lubrication http://en.wikipedia.org/wiki/Lubrication and acting as a humectant.
- Toothpaste, mouthwashes, skin care products, hair care products and soaps
- Solvent for flavours (such as vanilla) and food colouring
- Used in surface coatings and paints
- Used as a softener and plasticizer to impart flexibility, pliability and toughness
- Glycerol is the initiator to which propylene oxide/ethylene oxide is added

The production, consumption and usage figures of glycerol are indicated in Table 12.

Table 12 Production, Consumption, and Uses of Glycerol, 2001 (in thousands of tonnes).

	U. S.	Europe	Japan	Total
Annual capacity	169	315	59	543
Production	159	247	53	459
Consumption				
Personal/oral care products	75	46	15.5	136.5
Drugs/Pharmaceuticals	14	24	23	61
Foods/beverages	42	27		69
Polyether polyols	17	33	6	56
Tobacco	22	15	5	42
Alkyd resins	6	17	7.5	30.5
Other	13	79	29	121
Total	189	241	47.5	516

(Source: Chemical Economics Handbook)

Production of biofuels will have a significant impact on the prices commanded by glycerol. As a rough rule of thumb, about 1 kg of glycerol is produced for every 10 kg of fatty acid methyl ester. The relative value of glycerol may be expected to respond to market forces in a typical supply and demand pattern, i.e. as the availability of glycerol to the market increases, the demand, and consequently the price, will decrease. It has been estimated that capture of 5% of the total diesel market would result in the availability of an additional $1x10^6$ tonnes of glycerol, between 2 and 2.5 times the current world production[i]. The European biodiesel experience in the period 1997-2000 led to an almost 40% drop in the price of glycerol, with world prices demonstrating similarly large price fluctuations (Figure 6) illustrating a 50% fall in prices between 1995 and 1998. A reduction in the price of glycerol will have negative impacts on the economics of biodiesel production. The historical data indicates however that although there have been a number of major fluctuations in the market the general trend is nevertheless for a decrease in prices. Strongly rising fuel prices and falling tallow prices may offset some of these negative impacts[ii] (Figure 7).

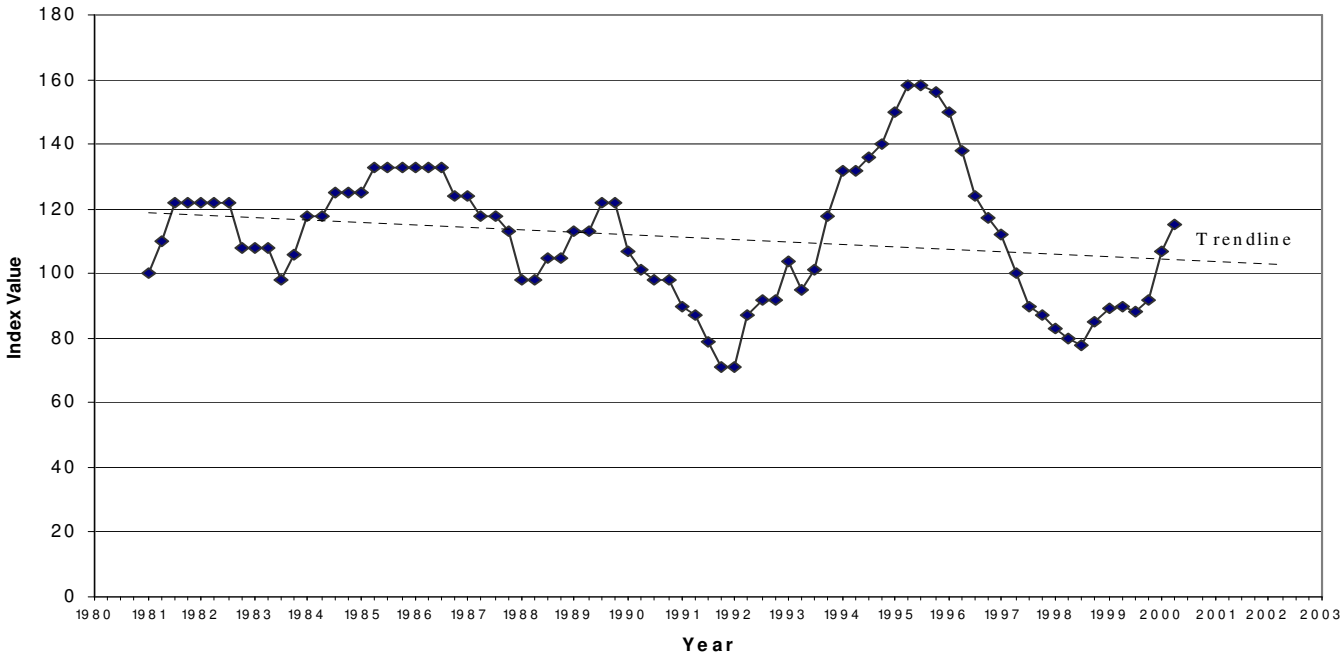

Figure 6 Historical Glycerol Price—99.5USP Grade (Index 100 = circa. $US 1100 / tonne. Source: Brunskill, A. "World Oleochemicals and Oil Prices – Cause or Effect".

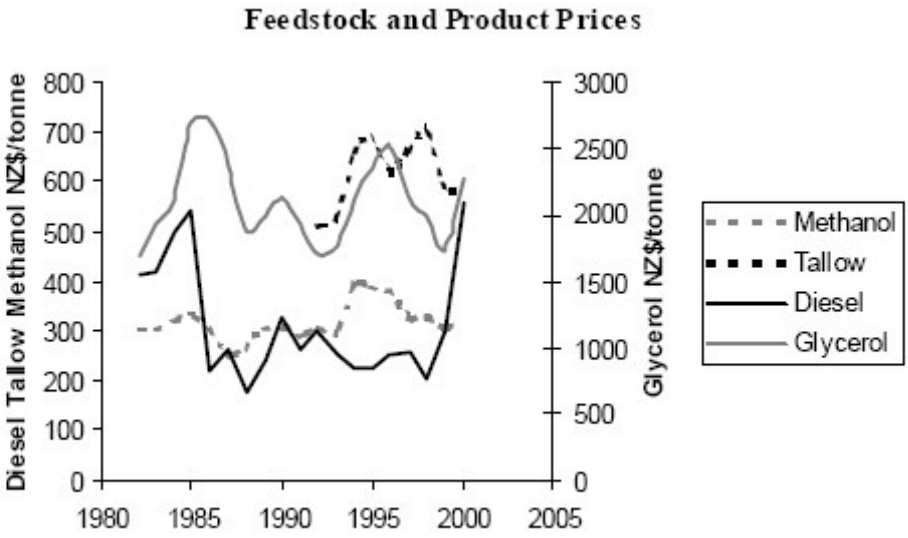

Figure 7 Costs of Biodiesel Production Source: Duncan J Energy Efficiency and Conservation Authority 2003

At
the present time, the biodiesel market has been moving ahead in Europe, but this increased consumption/demand has not been reflected in the market demand for glycerol. Consequently this has left the European glycerol sector with excess capacity of 10-20%. The rapid rise in biodiesel output will inevitably have a major impact on the oleochemicals

sector. To respond to this it is suggested that "Glycerol producers are seeking new product introductions based on glycerin in order to better balance supply/demand. New product introductions have been rare so far…"[iii]

Many of the current uses for glycerol are relatively small scale applications in which glycerol is used as an additive to the product. If and when biodiesel production increases the market for lower quality glycerol derived from tallow sources would be expected to fall for the supply and demand reasons described above. While the market for glycerol remains limited increasing the amounts available will lower the price for low grade material. It is possible that given an increased future supply of glycerol there may be the development of novel applications for glycerol use. Whilst glycerol production remains at current levels the development of novel applications will be limited. The development of new applications could however result in the uptake of glycerol from all sources, high and low grade thus stimulating the market. This would serve to transform the material from an increasingly low value by-product to a material that has sustained commercial value. There are several possible uses that have been identified and that are being researched which could absorb increased supply. These have been identified as:

1. **Selective oxidation of glycerol.** Glycerol's structure lends itself well to catalytic oxidative processes using inexpensive oxidizing agents such as air, oxygen, hydrogen peroxide or bleach. Combination of these inexpensive oxidizing agents with an inexpensive source of glycerol will allow the production of a number of new derivatives. A few research groups have investigated this technology, but focus has been limited to a small number of catalysts, leaving a number of questions of selectivity and yield unanswered.
2. **Glycerol carbonate as a new solvent and product**. Glycerol carbonate is a relatively new material in the chemical industry, but one that could offer some interesting opportunities, as it can be prepared directly and in high yield from glycerol. Glycerol carbonate has been investigated for several uses
   - as a novel component of gas separation membranes,
   - polyurethane foams as a surfactant component
   - as a new solvent for several types of materials,
   - as a component in coatings,
   - as a potential component of the paint industry,
   - acting as a non-volatile reactive solvent,
   - as a component of detergents.
3. **Glycerol as a component of new polymers**. Glycerol has traditionally played a role in the production of several types of polymers, some of which are available commercially. Selective esterification reactions can convert glycerol into polyglycerol esters, which have been suggested for use as biodegradable surfactants and lubricants and as replacements for conventional poly(oxoethylene) non-ionic surfactants.
4. **Biochemical transformations**. Glycerol can also serve as a feedstock in biochemical transformations. Glycerol has been investigated for the fermentative production of 1,3 propanediol, one of the primary components of DuPont's Sonora (1,3 PDO and terephthalic acid), a polymer being investigated for use in textiles and carpeting.

The quantities of glycerol produced from the process investigated here vary and are represented graphically in Figure 8. The glycerol produced was determined after

centrifugation and separation. When achieving higher tallow conversion efficiencies the amount produced approaches the expected ratio of 1 kg glycerol per 10 kg fatty acid methyl ester .

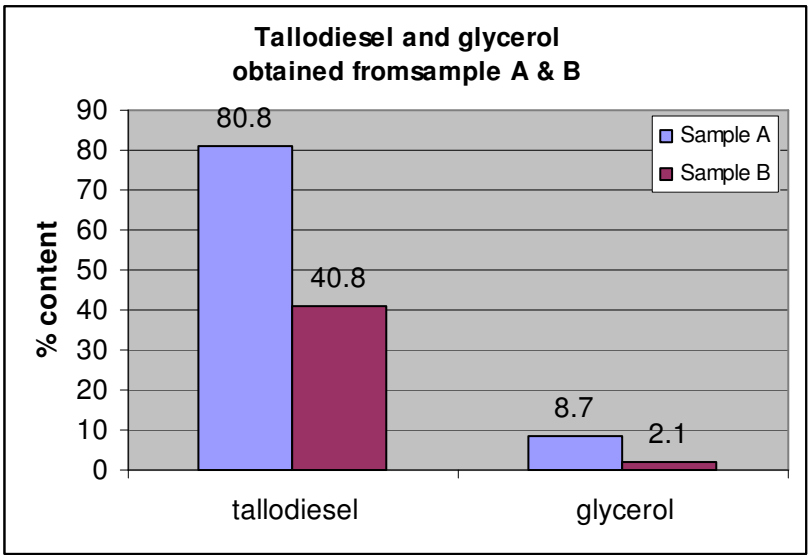

Figure 8 Tallodiesel and glycerol produced

As the conversion of diesel increases the amount of glycerol produced would be expected to increase similarly. This is reflected in the data from these investigations.

4.2. Unconverted fatty matter

Any unconverted fatty matter comprises of constituent parts of the original tallow and could find uses similar to those for which tallow is currently used after separation. Tallow is a natural product used mainly in foodstuffs, cosmetics and pharmaceuticals. Typical grades and uses include

- Edible Tallow - suitable for high grade soap without bleaching or for edible use.
- Top White Tallow - high grade tallow suitable, when bleached, for toilet soaps and used as is for laundry soaps. Titre > 40.5°C
- UK Grade 3 and Bleachable Fancy Tallow - Intermediate grades suitable for low grade toilet soaps or household soaps
- Low grade tallow - can be used straight for low grade soaps or bleached for better quality laundry soap. Titres from 35°C to 39°C.
- UK Grade 6 - FFA <20% but with titre >39°C. Suitable for low grade soaps.

Tallow has also been used at several locations as a fuel source with some success due to its similar energy content to conventional fossil fuels such as coal and oil. Although it costs more than other fuels, it does not contribute to carbon dioxide (greenhouse gas) build up.

5. TECHNO-ECONOMIC EVALUATION

The aim of this project is to determine whether via novel enzyme mediated alcoholysis, a protocol can be developed which can be used for the conversion of tallow into biofuel. Currently the UK Rendering Association estimates that over 200,000 tonnes of tallow are generated from animal carcass rendering annually, with 60 million tonnes of tallow/fats produced globally.

The key benefit of using enzyme mediated alcoholysis to convert the tallow into a biofuel is the conversion of the free fatty acid into a biofuel which is not possible using conventional chemical esterification. This is because the standard procedures liberate water, which can cause hydrolysis and saponification. This negatively affects the yield and recovery of the biodiesel.

Lipases under certain conditions can esterify and form glycerides from fatty acids and glycerol; they also catalyse the transesterification of fats, oils and free fatty acids with alcohol to form biofuel. This means that tallow with high levels of free fatty acid (such as animal by products) could be used to produce biodiesel.

In previous stages of the project tallodiesel yields of up to 80% have been achieved. Two other products are generated; glycerol and residual unconverted fatty matter. Most of the experimentation had been undertaken with an enzyme offer of £0.30 per kg of tallow, which was anticipated to yield a biodiesel of commercial viability. Attempts to reduce the enzyme offer at laboratory scale have been successful. Currently offers equivalent to £0.15 have been achieved although this has only been undertaken at laboratory bench scale. Agitation conditions have been shown to be a critical factor in achieving the conversion efficiencies obtained.

During leather processing excess fat and flesh is removed from the hide. This is unwanted matter which impedes the overall leather making process, therefore tanners take great pains to remove as much of this as possible. This excess matter, commonly referred to as “fleshings” is removed mechanically. The fleshings are collected and can follow alternative processing routes. In most cases the fleshings are a waste product for the tannery and so a cost is incurred due to the necessity of removing the material from the site. The current disposal route in these cases is land filling. However, as the charges for land filling increase the cost of this option will become increasingly prohibitive thus promoting the search for viable alternative disposal routes. Some tanneries are currently able to dispose of their waste fleshings in such a way that the costs of disposal are reduced. These disposal options currently include composting and tallow generation and have been adopted because the cost burden in the tannery is reduced. The disposal routes for waste fleshings can be summarised as follows:

1. Uncontrolled bovine wastes. These can be sent for disposal (landfill) and incur a cost, typically in the order of £70 per tonne of waste. Transport of the fleshings is a variable cost dependant upon the distance of the tannery from the disposal site. A typical average has been obtained from the leather industry in the region of £17 per tonne of waste.
2. Controlled bovine wastes are disposed of by an alternative route. Tanneries discharging waste generated from the processing of suspect cattle only pay a proportion of the costs of disposal; the rest is paid by the government under the Over-Thirty-Month (OTM) scheme. Essentially the cost of disposal is covered by the government and only the cost of transport is paid by the tannery. The cost of transport will again vary according to the proximity of the tannery to the disposal site. The cost of this transport is known to be in the order of £17 per tonne. It is anticipated that the OTM scheme will be brought to a close in the not too distant future and thereafter all fleshing wastes will be subject to disposal by the other disposal routes. All fleshing waste will have a potential conversion route to tallodiesel.

3. Uncontrolled bovine wastes can be composted

4. The fleshings can be rendered to result in the production of tallow.

5.1. Economics of fleshings disposal options.

The economics of the disposal options will be dependant upon several variables such as the prevailing land filling costs, tallow generation costs, tallow to tallow diesel conversion costs, and rate of excise duty on biodiesel. The overall process may also be affected by economies of scale. Small tanneries may find the cost of tallow generation uneconomic, the cost of the plant not being covered by the value of tallow generated. Larger tanneries producing substantial quantities of fleshings may find that not only can the cost of tallow generation be justified but that on-site conversion to tallodiesel is also feasible. In examining the economics of the possible options certain preliminary assumptions will be made. The initial starting point will be to work on the basis that a typical European sized tannery processes 15,000 hides per week.

5.2. Quantity of fleshings produced

A tannery processing 15,000 x 32 kg hides per week would process 480 tonnes of hides per week. Tannery records indicate that these production levels will generate approximately 100 tonnes of fleshings per week (this is a conservative figure – some tanners report higher amounts of flesh removal to an amount approaching 180 tonnes for this amount of hides). Assuming a 50 week working year this results in the generation of 5,000 tonnes of fleshings annually.

5.3. Fleshings disposal routes

5.3.1. Fleshings Disposal Route 1 – Landfill

In the absence of any other treatment options the fleshings could be simply disposed of to landfill. The current cost of disposal is £70 per tonne of fleshings disposed provided the fleshings originate from uncontrolled hides. Transport cost must be added to this figure and will vary; £17 per tonne is a realistic average value. Part of the charge consists of landfill tax. The charge was £15 per tonne from 1st April 2004 with a £3 increase per tonne in 2005/6. The Treasury has confirmed that, post-2005/6; the charge will rise by “at least” £3 per tonne in subsequent years to a medium to long-term level of £35 per tonne.

Annual disposal of 5,000 tonnes fleshings would therefore cost:-
£ 360,000 disposal + £75,000 landfill tax = -£435,000 at current rates
£ 360,000 disposal + £175,000 landfill tax = -£535,000 by 2012
These are minimum level costs, as there is reduced option to landfill, i.e. with fewer sites, costs will increase for disposal in addition to tax

5.3.2. Fleshings Disposal Route 2 – Composting

Provided that the disposal route exists a similar option is composting. This is a method in current practise but not one that is available to all tanners. For the tanner the disposal

route is not dissimilar to landfill. The costs of disposal by this route are similar to landfill but avoid the costs associated with the landfill tax.

Annual disposal of 5,000 tonnes fleshings
Current cost £ 360,000 disposal (2005)

5.3.3. Fleshings Disposal Route 3 – Tallow production

If the fleshings are rendered to yield tallow there are three onward disposal routes that may be followed

- Incineration to yield energy
- Sale as a raw material for a variety of manufactured products
- Conversion to biodiesel

Regardless of the onward disposal route preliminary preparation of tallow is required. Tanners processing fleshings to tallow have determined that the products of the tallow generation process can be characterised, after mechanical separation, into three components, tallow, greaves and water. Mechanical separation would be the preferred method of production if subsequent conversion to tallodiesel is anticipated. Acid cracking of the tallow will result in a sulphur content in excess of the limits required of diesel fuel, and so subsequent treatment would be required to remove this.

1 tonne of wet fleshings when rendered yields 250 kg tallow
400 kg greaves
350 litres water

In order to produce tallow the following process is carried out:-

Fleshings sent to a macerator

The fleshings removed from the skins during the fleshing operation fall from the fleshing machine to a conveyor that transports them to the macerator. The costs of this operation are immaterial as the fleshings need to be transported away from the machine regardless of the disposal method. Maceration is an essential step to reduce the fleshings in size and to commence breakdown of the fleshy and fatty matter. The fleshings obtained from the hides are often several centimetres long and would readily foul process equipment if not reduced in size.

Macerated fleshings sent to collection hopper

After maceration the fleshings are transferred to a collection hopper from where they are transferred via pump to a rendering tank.

Collected and re-macerated fleshings rendered in one of two holding tanks

The macerated fleshings are heated over a period of 20 to 30 minutes to mobilise the tallow and render the material into its separate components (water, tallow, and greaves).

Heated (rendered) matter sent to centrifuge

The rendered matter is centrifuged to complete physical separation into the three component products.

a. Contaminated water. The water may be discharged to an effluent treatment plant. Most tanneries have their own effluent treatment capacity and this therefore represents negligible cost. The water has a high COD content (in excess of 20,000 mg /litre). This could lead to cost implications if the fleshings were to be rendered at an alternative site which required the construction of treatment facilities, or if the effluent were to be discharged, untreated, into the municipal system.
b. Greaves. Protein and solids separated out during centrifugation are referred to as greaves. These are largely solid although there is some moisture associated with them. The greaves could be dried to reduce weight prior to transport for landfill disposal or further rendering. This would involve additional costs. In the absence of a requirement to dry them they are transported and disposed of in the form recovered from the centrifuge.
c. Tallow. The tallow is pumped off into a collection tank and periodically tankered to a customer. The holding tanks and tankers must be maintained at a constant temperature to ensure continued mobility of the liquid tallow.

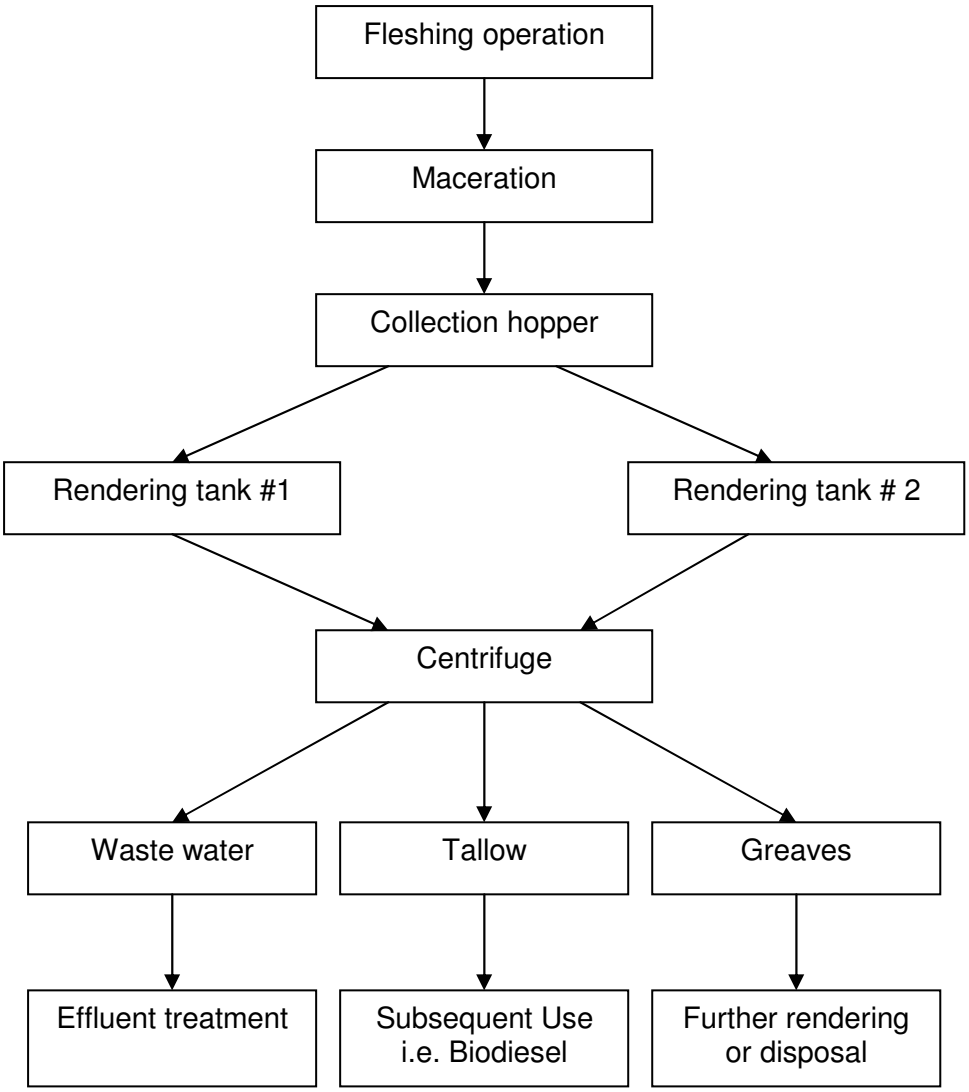

Figure 9 Fleshings to tallow production flow

From the process described it is possible to determine the approximate costs of operating a tallow recovery plant within the tannery. The figures are based on a medium sized tannery generating 100 tonnes of fleshings per week.

Table 13 Costs of tallow recovery

	Initial cost	**Annual cost**
Tallow recovery plant of 100 tonne/week fleshings handling capacity	£ 260,000	£26,000
Operations (energy)	Not applicable	£7,500
Operations (manpower)	Not applicable	£10,000
Maintenance	Not applicable	£15,000
Total annual costs		£58,500

5.4. Tallow uses

5.4.1. Boiler fuel

Recovered tallow could be incinerated to partly replace boiler fuel. This is an option that has been used in the past by tanneries. The introduction of the Waste Incineration Directive which comes into effect in January 2006 will cause this to be an uneconomic option due to the costs associated with conforming to the regulations required by this directive. The fuel replacement value of the tallow incinerated by this method is £70 per tonne of tallow. The fuel replacement value will depend upon the cost and energy content of the fuel being replaced by tallow. Greaves disposal represents a cost of £70 per tonne with additional transport costs of about £17 per tonne.

Annual disposal of 2,000 tonnes greaves	(£174,000)
Annual fuel value of 1,250 tonnes tallow	£87,500
In-tannery waste water treatment costs	£0
Annual tallow recovery plant costs	(£58,500)
Total (cost)/benefit	(£145,000)

Although this figure represents a cost to the tannery, in comparison to the landfill option (cost =£435,000) it represents a reduction in cost. As indicated above due to the modifications that would be required of a tannery in complying with the Waste Incineration Directive, in 2006 this will no longer be considered to be an economic option.

5.4.2. Chemical industry base product

Recovered tallow could be sold as a base product for the chemical industry. This option is currently available to tanners. The value of the tallow varies considerably with prices ranging from £0.07 per kg to £0.10 per kg being offered. Greaves disposal represents a cost of £70 per tonne with additional transport costs of about £17 per tonne.

Tallow sale value	£70/tonne	***£100/tonne***
Annual disposal of 2,000 tonnes greaves	(£174,000)	***(£174,000)***
Annual sale value of 1,250 tonnes tallow	£87,500	***£125,000***
In-tannery waste water treatment costs	£0	***£0***
Annual tallow recovery plant costs	(£58,500)	***(£58,500)***
Total benefit/(cost)	(£145,000)	***(£107,500)***

5.4.3. Biodiesel

Recovered tallow could be converted to tallodiesel and used as a fuel. The tallow recovery plant described above would be used in the initial generation of the tallow. The tallow produced would need to processed to biodiesel

Biodiesel plant

The production of biodiesel will be relatively simple. Holding tanks will be required for the tallow, methanol and enzyme. The tallow holding tank will need to be kept heated to ensure that the tallow remains mobile. Holding the tallow at 40^{o}C would keep it at the temperature required for the reaction.

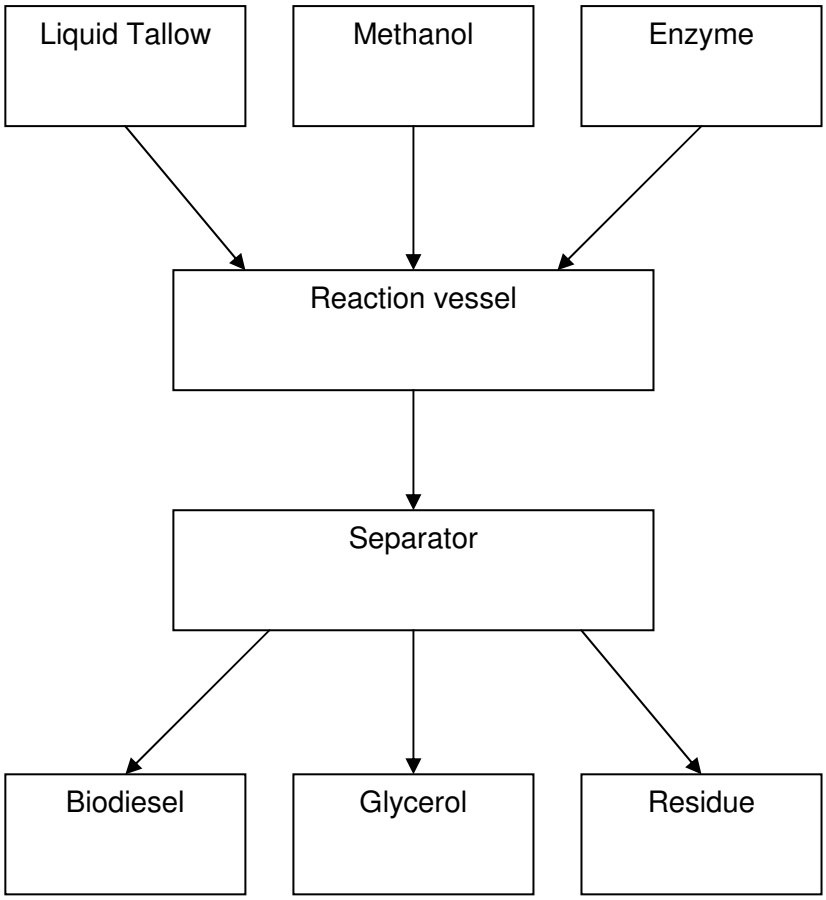

Figure 10 Biodiesel production flow

The three feedstocks are pumped to the reaction vessel at a controlled rate where they are agitated for 48 hours at 40^{o}C. The products are transferred to a tricantor for separation and purification

Biodiesel plant operating within the tannery
Capacity basis:
A 25 tonnes of tallow per week
B 75 tonnes of tallow per week

Table 14 Costs of biodiesel production

	Initial cost 25 tonne/week capacity	**Annual cost 25 tonne/week capacity**	**Initial cost 75 tonne/week capacity**	**Annual cost 75 tonne/week capacity**
Biodiesel plant construction	£ 100,000	£10,000	£ 150,000	£15,000
Operations (energy)	Not applicable	£7,500	Not applicable	£10,000
Operations (manpower)	Not applicable	£ 10,000	Not applicable	£ 10,000
Maintenance	Not applicable	£ 15,000	Not applicable	£ 15,000
Total annual costs		£ 42,500		£50,000

Greaves disposal represents a cost of £70 per tonne with additional transport costs of about £17 per tonne.

Fuel duty
In the UK 2005 budget it was indicated that the Government's policy is that fuel duty should rise at least in line with inflation as it seeks to meet its targets of reducing polluting emissions and funding public services. Main road fuel duties will increase in line with inflation by 1.22p per litre. Owing to the sustained volatility in the oil market, the changes in rates will be deferred until 1 September 2005. And the planned duty differentials for biofuels and road fuel gases will continue until 2007-08, consistent with the Government's alternative fuels framework.

Table 15 UK Fuel Duty

Pence per litre (unless stated)	**Old duty rate**	**Change**	**New duty rate**
Sulphur-free petrol/diesel	47.1p	+1.22p	48.32p
Ultra low sulphur petrol/diesel	47.1p	+1.22p	48.32p
Biodiesel	27.1p	+1.22p	28.32p
Bioethanol	27.1p	+1.22p	28.32p

SCENARIO 1 Medium sized tannery
Enzyme offer required £0.30/kg tallow

Tallodiesel production quantity 1,250 tonnes per year

Chemicals required for tallodiesel production

Tallow	1 tonne
Enzyme	8.6 kg
Alcohol (methanol)	250 litres

Enzyme offer calculated as 30p enzyme per kg tallow

Cost of alcohol/tonne	£200
Value of tallow/tonne	£70

For 1 tonne tallow	£70
250 litres alcohol	£50
Enzyme	£300
Total	£420

Diesel price ~ £0.98/litre (2005) includes excise duty at £0.2832 per litre
Density of diesel 0.88 kg/l
1 tonne tallodiesel = 1,136litres tallodiesel.

1136 litres regular diesel	£1113 inc duty
1136 litres tallodiesel	£420 + (£0.2832 x 1136 = £322) = £ 742

Balance of revenue less cost = £371/tonne

Annual disposal of 2,000 tonnes greaves	(£174,000)
Annual value of 1,250 tonnes tallodiesel	£463,750
In-tannery waste water treatment costs	£0
Annual tallow recovery plant costs	(£58,500)
Annual biodiesel plant costs	(£42,500)

Annual total benefit	+£188,750

SCENARIO 2 Large sized tannery
Enzyme offer required £0.15/kg tallow
Tallodiesel production quantity 3,750 tonnes per year

Chemicals required for tallodiesel production

Tallow	1 tonne
Enzyme	17.2 kg
Alcohol (methanol)	250 litres

Enzyme offer calculated as 15p enzyme per kg tallow

Cost of alcohol/tonne	£200
Value of tallow/tonne	£70
For 1 tonne tallow	£70
250 litres alcohol	£50
Enzyme	£150
Total	£270

Diesel price ~ £0.98/litre
Density 0.88 kg/l
1 tonne tallodiesel = 1,136litres tallodiesel.

1136 litres regular diesel £1113 inc duty
1136 litres tallodiesel £270 + (£0.2832 x 1136 = £322) = £ 592

Balance of revenue less cost = £479/tonne

Annual disposal of 6,000 tonnes greaves	(£522,000)
Annual value of 3,750 tonnes tallodiesel	£2,220,000
In-tannery waste water treatment costs	£0
Annual tallow recovery plant costs	(£58,500)
Annual biodiesel plant costs	(£50,000)
Total benefit	£1,589,500

In the above two scenarios the potential income from the sale of recovered glycerol has not been included. The market for glycerol is limited and it may be assumed that as the volume of biodiesel produced globally increases the price of glycerol will fall. Furthermore there would be cost associated with glycerol purification. It is assumed that revenues and costs associated with generation and disposal of low grade glycerol will be neutral.

6. LIFECYCLE ANALYSIS

The Society of Environmental Toxicology and Chemistry (SETAC) originally defined LCA as: "An objective process to evaluate the environmental burdens associated with a product, process, or activity by identifying and quantifying energy and materials used and wastes released to the environment, to assess the impact of those energy and materials uses and releases on the environment, and to evaluate and implement opportunities to affect environmental improvements. The assessment includes the entire life cycle of the product, process, or activity, encompassing extraction and processing of raw materials, manufacturing, transportation and distribution, use/re-use/ maintenance, recycling, and final disposal."[iv]

A full lifecycle assessment of tallodiesel is beyond the scope of this project and is worthless until the methodology for industrial scale production has been fully established. Nevertheless valuable indications can be obtained from a review of LCAs of diesel and biodiesels undertaken to date.

Lifecycle analyses of biodiesel have been carried out in a number of previous studies indicating that biodiesel differs from petroleum diesel in several ways. There are no standard criteria for performing a lifecycle analysis; consequently the methodologies and parameters used in determining inputs and outputs are not uniform. This makes direct comparison between the studies invalid, but broad conclusions may be drawn.

In May 1998 the US Department of Energy and the US Department of Agriculture published an extensive comparative study[v]. The results can be summarised as follows:

Total energy efficiency ratio
(total fuel energy/total energy used in production, manufacture, transportation and distribution)

Petroleum diesel	**80.55%**
Biodiesel	**83.28%**

Total fossil energy efficiency ratio
(total fuel energy/total fossil energy used in production, manufacture, transportation and distribution)

Petroleum diesel	**321%**
Biodiesel	**83%**

Fuel economy

Petroleum diesel	**131,295 Btu/gal**
Biodiesel	**117,093 Btu/gal**

Emissions of carbon dioxide
Overall lifecycle emissions of carbon dioxide from biodiesel are 78% lower than from petroleum diesel

Emissions of carbon monoxide
Overall lifecycle emissions of carbon monoxide from biodiesel are 35% lower than from petroleum diesel

Emissions of Total Particulate Matter
Overall lifecycle emissions of total particulate matter from biodiesel are 32% lower than from petroleum diesel

Emissions of sulphur oxides
Overall lifecycle emissions of sulphur oxides from biodiesel are 8% lower than from petroleum diesel

Emissions of methane
Overall lifecycle emissions of methane from biodiesel are 3% lower than from petroleum diesel

Emissions of nitrogen oxides
Overall lifecycle emissions of nitrogen oxides from biodiesel are 13% greater than from petroleum diesel

Emissions of hydrocarbons
Overall lifecycle emissions of hydrocarbons from biodiesel are 35% greater than from petroleum diesel

Emissions of wastewater
Overall lifecycle emissions of wastewater from biodiesel are 79% lower than from petroleum diesel

Emissions of hazardous solid wastes
Overall lifecycle emissions of hazardous solid wastes from biodiesel are 96% lower than from petroleum diesel

Emissions of non-hazardous solid wastes
Overall lifecycle emissions of non-hazardous solid wastes from biodiesel are 100% greater than from petroleum diesel

In a study conducted at The Institute for Resource Efficient and Sustainable Systems,[vi] a Lifecycle study of biodiesel from tallow and used vegetable oil has been undertaken. In this study the results were based on the ecological footprint of the biodiesels produced in comparison to fossil diesel. The study examined biodiesel production as assessed within several system boundaries, from the rendering process, from the slaughtering stage and even including production of cattle fertilisers and fodder. Only that data relating to the rendering stage need to be referred to since this is the stage at which tannery wastes would be equivalent.

The starting point for the Life Cycle Assessment was the transesterification process. Its inputs were classified as energy, process chemicals and raw material (fat after rendering or collected waste cooking oil). Together with the environmental impacts of these inputs, the impacts of the combustion of biodiesel and the transport (collection of raw material and fuel delivery) were assessed. In this assessment the footprints for the inputs of the esterification process, process chemicals, combustion and transport are the same for biodiesels produced from waste cooking oils and from tallow. The assessment was based on biodiesel produced by a chemical esterification process, however the impact and

footprints associated with the use of potassium hydroxide and sulphuric acid were considered to play only a minor role due to their function as catalysts.

The ecological footprints were ultimately determined as given in Table 16. (Ecological Footprint Analysis measures the amount of renewable and non-renewable ecologically productive land area required to support the resource demands and absorb the wastes of a given population or specific activities).

Table 16 Ecological Footprint of Biodiesel

	Ecological footprint M^2A/MJ
Combustion processes	2.03
Fuel delivery (50km)	0.30
Transport to esterification (100 km)	0.54
Energy	0.86
Process chemicals	1.06
Sub Total	**4.78**
Biodiesel from used vegetable oil	**4.78**
Rendering process	1.82
Biodiesel from tallow	**6.60**
Diesel from fossil fuel	**26.1**

A report by Rollefson, Fu and Chan[vii] included a lifecycle analysis of the entire biodiesel cycle. The total emissions through all stages of diesel and biodiesel production according to the different sources for the two processes were assessed (Table 17). Included within the biodiesel emissions were the same estimates for storage, distribution and dispensing as used for diesel. In this study a simple mass allocation system was used to account for the co-products of glycerol and protein meal.

Table 17 Inputs & Outputs of Biodiesels

Inputs (g/MJ $_{out}$)	Diesel	Biodiesel yellow grease	Biodiesel tallow	Biodiesel soybean	Biodiesel canola
Crude oil	72.3	1.4	2.3	3.3	3.6
Hard coal		0.3	0.3	0.4	0.5
Lignite		0.7	0.7	0.7	0.7
Natural gas		7.5	16.3	10.4	13.7
Inert rock		6.9	7.3	7.4	7.2
Sodium chloride		1.1	1.1	1.1	1.1
Phosphorous minerals				3.9	5.1
Waste cooking oil (405 water)		120.5			
Fat (slaughterhouse residues)			200.8		
Emissions (g/MJ $_{out}$)					
Carbon dioxide	290203.5	24311.1	25976.5	32616.5	34037.5
Carbon monoxide	729.1	-96.3	-89.1	-83.6	-78.9
Nitrogen oxides	2356.5	1467.9	1479.0	1506.4	1548.2
Nitrous oxide	3.8	0.7	0.8	55.93	145.95
Sulphuric acid	50.0	12.0	12.0	12.0	12.0
Methane	787.8	62.5	114.3	91.0	119.6
Particles to air	39.6	11.9	12.4	15.4	16.3
Non-methane hydrocarbons	147.9	7.3	24.7	85.3	171.9

The studies indicate firstly that the environmental impact of biodiesel is significantly lower than that for petroleum derived diesel. This is irrespective of the feedstock material used for biodiesel production. The Rollefson study demonstrates also that emissions from tallow derived biodiesel compare favourably with those from plant derived biodiesel, giving rise to reduced emissions for most substances measured.

The lifecycle analysis of an enzyme mediated transesterification process for the production of biodiesel will return different values compared to those of a traditionally produced tallow derived diesel. The differences are likely to be principally associated with the LCA of the enzyme; however, given that the enzyme is acting as a catalyst (even though consumed in the process) the amount of enzyme consumed is small and is unlikely to alter the values of a tallodiesel significantly. Data concerning the LCA of the enzyme is limited to the following:

Assuming that the dose rate at which the enzyme is used is approx 5 kg enzyme per tonne of tallodiesel, the energy input required for the manufacture of the enzyme is

approximately 7.5 Kilo Watt Hours per tonne of tallodiesel, (1.5 Kilo Watt Hours per kg of enzyme)

Waste generated from enzyme production is approximately 1.25 kg (wet weight) of biomass requiring landfill and 11.25 kg of liquid waste (of similar BOD to sewage) requiring treatment prior to disposal per tonne of tallodiesel. (0.25 g wet weight of biomass per kg of enzyme) (2.25 kg liquid for disposal per kg of enzyme).

7. CONCLUSIONS

The analysis of the tallodiesel obtained at earlier stages in the project indicate that the conversion efficiency is dependant upon the enzyme used. The earlier analysis by ester type also indicated some exceptionally high conversion efficiencies, although this was attributed to possible settling of the residual tallow solids prior to sampling. The current work has provided further evidence that the agitation conditions of the reaction are a key issue in determining whether successful, efficient conversion can be achieved. Yields of at least 80% were obtained in the initial laboratory analysis and have been replicated in larger lab-scale experimentation. Currently yields of 60% are the best that have been obtained in the bulk (100kg tallow) experimentation.

When good conversion is achieved the products can be readily separated into three phases – tallodiesel, glycerol and residual fatty matter. The diesel and glycerol have possible marketable value, the unconverted fatty matter does not. An unknown at this stage is whether the unconverted fatty matter could also be converted to tallodiesel and glycerol, either by increased enzyme application rates or by enhanced mechanical action, or whether this remains a component that will require an alternative disposal route. The fatty matter residue does have a final possible use as a base material in the production of low grade soaps.

The Techno-economic calculations indicate that even for medium sized tanneries using higher than anticipated enzyme offers economic conversion of tannery waste products via tallow to biodiesel is feasible. The current method of disposal to landfill incurs costs whereas the conversion to biodiesel has the potential for revenue generation.

The typical calculated costs of disposal in summary are

Landfill	(£435,000)	at current rates
	(£535,000)	anticipated 2012 rates
Composting	(£360,000)	
Tallow incineration	(£145,000)	
Chemical feedstock	(£107,500)	
Biodiesel (medium sized tannery)	£136,250	
Biodiesel (large sized tannery)	£1,533,250	

It would be feasible for tanners of sufficient production capacity to produce the biodiesel themselves. Tanneries operating fleets of vehicles may well be able to fuel their fleet using the biodiesel produced from their own waste products. Conventional biodiesel is a blend of 20% biodiesel and 80% petroleum derived diesel. Alternative outlets may need to be found if a blend continues to be used.

Smaller sized tanneries are unlikely to be able to justify construction of on-site tallow production and biodiesel production equipment. However, given the potential positive benefits of biodiesel conversion the future possibility of transfer of tannery wastes from smaller tanneries to larger processing plants may well be economically viable.

REFERENCES

[i] Sonntag, N. O. V. in Glycerine. A Key Cosmetic Ingredient, Jungermann, E.; Sonntag, N. O. V., eds., Marcel Dekker, New York, Chapter 9 (1991

[ii] Brunskill, A. "World Oleochemicals and Oil Prices – Cause or Effect".
Asean Oleochemicals Manufacturers Group. 2001.

[iii] Chemical Economics Handbook, "Glycerol".

[iv] Fava, J., Denison, R., Jones, B., Curran, M. A., Vigon, B., Selke, S. and Barnum, J. (eds) (1990) *A Technical Framework for Life Cycle Assessments*, Report of the workshop organised by the Society of Environmental Toxicology and Chemistry, Smugglers Notch, Vermont, USA.

[v] John Sheehan, J. Camobreco, V. Duffield, J. Graboski, M & Shapouri, H *An Overview of Biodiesel and Petroleum Diesel Life Cycles* National Renewable Energy Laboratory, May 1998

[vi] Niederl, A. & Narodoslawsky, M *Life Cycle Assessment – study of Biodiesel from Tallow and Used Vegetable Oil* Institute for Resource Efficient and Sustainable Systems Graz, December 2004

[vii] Rollefson, J. Fu, G. & Chan, A *Assessment of the Environmental Performance and Sustainability of Biodiesel in Canada* Natural Resources Canada, November 2004