Biodiesel: a microbiologist's perspective

Stuart Shales discusses the complex issues surrounding the use of biofuels from a microbiologist's perspective

The subject of biofuels is very topical and features heavily in the news media. This coverage has increased considerably since the publication of the 'Stern Report' earlier in 2007 (Stern, 2007). In his review Sir Nicholas Stern discusses the economic impact of climate change and proposes strategies for investment to mitigate the effects of carbon dioxide (CO_2) accumulation in the atmosphere and resultant climate change.

In 2005 in the UK, 38.1 million tonnes of fuel was used for motor transport, both freight and domestic (source: DTI). Of this, 18.7 million tonnes was petrol and 19.4 million tonnes was diesel (DERV). During the period 2004 to 2005, diesel consumption exceeded petrol for the first time in the UK, much of this being due to the high sales of new diesel cars for the domestic sector. This high consumption of crude oil derived fuels for motor transport together with that for rail, marine, aviation and power generation has a major impact on CO_2 emissions in the UK. One of the drivers for the introduction of renewable, CO_2 neutral fuels is the need to mitigate greenhouse gas emissions. There are also other drivers, including the security of fuel supply. It is predicted that workable oil reserves will peak in the next two or three decades at a time when demand for oil is growing dramatically, as nations such as China and India become more wealthy and car ownership increases. For the UK there is another related issue: until recently as a result of North Sea Oil production the UK, there was a net export of crude oil, however the North Sea reserves are dwindling and the UK is now a net importer of crude oil.

The UK Government has introduced a Road Transport Fuels Obligation which seeks a 5% substitution of motor fuels with renewable sources by 2010. On top of this, the EU has a target of 10% substitution by 2020. So what are the options for renewable fuels? Currently the principal biofuels are bioethanol and biodiesel — first generation biofuels — as substitutes for petrol and diesel respectively. One of the main aspects of recent press coverage concentrates on concerns relating to the competition of agricultural land use for food production versus biofuel production. This gives rise to the question "is there sufficient land for both?" The answer to this question is "no", particularly if all motor fuels are to be derived from renewable sources. To this end, second generation biofuels produced from plant biomass will become increasingly important.

The Microbiological Perspective

What role does microbiology play in biofuel production? For bioethanol the answer is immediately apparent as ethanol is produced by fermentation of sugars by yeast and some bacteria. The production of alcohol by yeast such as *Saccharomyces cerevisiae* has been used for several thousands of years in the formation of alcoholic beverages and now this technology is being applied to biofuel production. The Brazilian alcohol programme has been in operation since the early 1970s and now nations such as the United States are developing extensive alcohol production facilities (see page 30 for more details). In addition to bioethanol as a petrol substitute, there is growing interest in the production of biobutanol which has an energy density virtually the same as that of petrol, whereas that of ethanol is considerably less. Thus fuelling a car with butanol will provide more miles per gallon than ethanol. Biobutanol is produced anaerobially by some *Clostridium* species and has been undertaken commercially in the past.

This article will concentrate on microbiological aspects of first generation biodiesel production and its use. Finally it will discuss some aspects of microalgal derived biodiesel fuels.

Biodiesel

Biodiesel that is currently used as a substitute for diesel fuel is derived from vegetable oils. In the UK and much of Europe the main source of vegetable oil is oilseed rape, *Brassica napus*, (figure 1a) although in southern, warmer countries the sunflower, *Helianthus annuus*, (figure 1b) can be grown for this purpose. A hectare of oilseed rape will yield approximately 1,000 kg of oil whereas sunflowers yield 800 kg of oil (Tickell, 2000). There are other oil producing plants grown for biodiesel production outside Europe, notably *jatropha* and, controversially, the oil palm. The latter has the highest yield of oil, 5,000 kg per hectare, but there are environmental concerns regarding the destruction of tropical rain forests that is taking place to grow oil palm crops. Furthermore, biodiesel produced from palm oil tends to gel at winter temperatures and thus can only be used in dilute blends of diesel fuels.

Figure 1a. Oilseed Rape

Pure vegetable oils cannot be used directly as diesel fuels in most motor vehicles. Oils are comprised predominantly of triglycerides (triacylglycerols) and these have a relatively high molecular weight which causes them to be too viscous. Furthermore, gums present in pure vegetable oils can accumulate in fuel injection components and may lead to failure. Instead, pure vegetable oil is processed by transesterification to biodiesel which is essentially a mixture

Figure 1b. Sunflower

of fatty acid methyl esters (FAMES). This process is illustrated in figure 2. The process uses methanol as a co-substrate and commercially it is usually catalysed using sodium hydroxide or potassium hydroxide. It is essential that no water is present otherwise soap may be produced and this will contaminate the biodiesel. Under some circumstances acid catalysts may be used. This normally uses sulphuric acid - nitric acid should be avoided as its presence may lead to the production of nitroglycerine! Acid catalysis is considerably slower but may be used if recovered vegetable oil (used cooking oil) is the starting material.

In Europe there is a quality standard, EN14214 for commercial biodiesel. A by-product of the process is glycerol (glycerine); this could have commercial value for the cosmetics industry but unfortunately tends to be contaminated with alkali and other products. Clean-up costs are prohibitively high so most of the glycerol is disposed of, for example by anaerobic digestion to biogas which in turn can be used as a fuel/energy source. In some instances the glycerol can be combusted for heat and power production.

One area of research interest has been the development of alternative catalysts for the transesterification process and for microbiologists this has involved developing enzyme catalysts (Shimada *et al.*, 2002). The potential advantage of using enzymes is that they can be immobilized and hence reused. It is also possible to develop a continuous process. Furthermore, the glycerol by-product will be cleaner, not contaminated with alkali, thus increasing its commercial value. The enzymes used are lipases from a variety of microbial sources including *Candida antartica*, *Rhizomucor miehei* and *Pseudomonas cepacia* (Shimada *et al.*, 2002; Salis *et al.*, 2005; Noureddini *et al.*, 2005). The use of microbial lipases as biocatalysts for the transesterification process is a challenge to biotechnologists. Firstly, the reaction is two phase, as the two products, biodiesel (FAME) and glycerol, are immiscible and separate out during the process. Secondly, the methanol used as a co-substrate is toxic to lipase enzymes and its presence at high concentration may inhibit the transesterfication process. One solution to this toxic effect is to add the methanol sequentially in a fed-batch system. Thirdly, the process must be undertaken in the absence of water and the plant oils are by nature hydrophobic.

Whether or not enzyme catalysts are used commercially will depend on these technical hurdles being overcome. Furthermore, the alkali catalysts are very inexpensive and the reaction rate is very fast. Thus enzyme catalysts would need to offer distinct advantages if they were to be used.

Another microbiological aspect of biodiesel is its environmental impact. Conventional hydrocarbons such as diesel fuel can have a detrimental effect when accidentally discharged into the environment. The environmental impact of biodiesel has been investigated. Lapinskiene *et al.*, (2006) demonstrated that in non-adapted, aerated soil biodiesel had

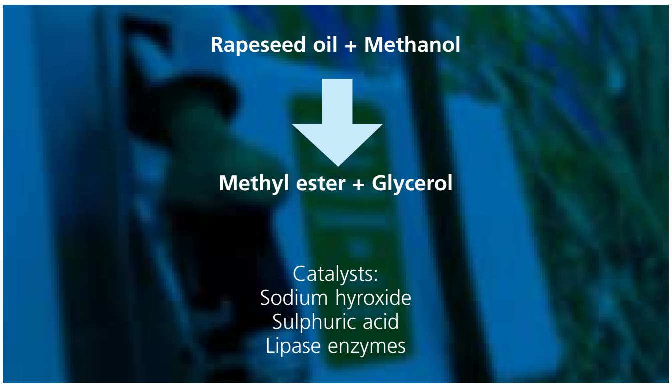


Figure 2. Biodiesel production by transesterification

no toxic effect up to 12% (w/w) when compared to diesel which had toxic effects at above 3% (w/w). Additionally, biodiesel was rapidly transformed and degraded in the soil whereas diesel was more resistant to degradation. The reduced ecotoxicity and rapid biodegradation of biodiesel may be of importance in its utilization in environmentally sensitive areas; an example of its use could be that of marine fuel for boats using the Norfolk Broads, canals and other waterways.

Microalgal Diesel Fuels

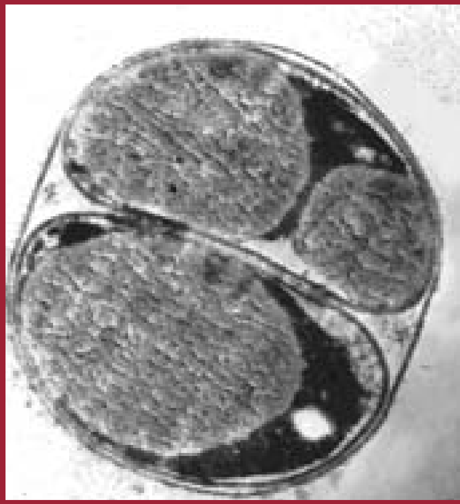
There has been considerable interest in the development of microalgal diesel fuels (Chisti, 2007). One of the main drivers for this is the higher photosynthetic efficiency of microalgae when compared to plants and hence the potentially higher productivity per unit area. The National Renewable Energy Laboratories in the United States undertook a major research programme in this area from the late 1970s through to the end of the 1990s (NREL, 2005). The programme included microalgal hydrocarbon and lipid production, as well as investigating different culturing conditions. One of the conclusions made was that although it is technically possible for microalgae to produce biodiesel fuels, the cost of extracting products for the cells was prohibitively expensive. Also microalgae need lagoons or photobioreactors for their culture which involves capital costs. At the University of the West of England a somewhat different approach has been adopted (Scragg *et al.*, 2003). Systems have been developed to use the complete algal cell as a particulate diesel fuel. *Chlorella vulgaris* was grown in a 230 litre tubular photobioreactor (figure 3). The cells are 5μm in diameter (figure 4) which is suitable for injection into a diesel engine. Algal cells in suspension with the addition of a surfactant, Triton-X100, formed a stable emulsion with diesel fuel or biodiesel, which acts as a carrier and preliminary results illustrated that these emulsions were combusted successfully in a modified diesel engine. The purpose of this work was the development of fuels for static diesel engines rather than fuel for motor vehicles. The algae could be grown using photosynthetic energy or heterotrophically on an organic waste stream.

Figure 3. 230 litre tubular flow bioreactor for culturing microalgae

Conclusion

First generation biofuels, bioethanol and biodiesel, offer an opportunity to substitute in part current motor fuels. However, the land required to produce these fuels in the UK is limited. It is quite feasible that the UK can produce sufficient first generation biofuels to meet the requirements of the Government's Road Transport Fuels Obligation (RTFO) and possibly the EU's target for 10% fuel substitution by 2010. To go beyond these targets, advanced second generation biofuels will be required and microalgal biodiesel may be an option. Another option is Fischer-Tropsch oil, produced by gasification of plant biomass followed by Fischer-Tropsch catalysis to produce synthetic hydrocarbons. To read further on this subject, please see Hamelinck & Faaij (2006) who discuss the outlook for a wide range of advanced biofuels.

Figure 4. Chlorella vulgaris (cell diameter = 5μm)



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