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Research review paper

Biocatalysis: Towards ever greener biodiesel production

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ABSTRACT

The cost of lipases and the relatively slower reaction rate remain as the major obstacles for enzymatic production of biodiesel as opposed to the conventional chemical processes. This paper reviews the starting oils usually employed in biodiesel production, the processes for transforming them to biodiesel placing particular emphasis on enzymatic transesterification. The pros and cons of the lipase-based process, the key operational variables and the technological alternatives for attenuating lipase deactivation are also discussed. Finally, suggestions are made for future studies, paying particular attention to the use of whole cell immobilization in the production process, as this methodology may reduce both the cost of the biocatalyst and dependence on lipase manufacturers.

Contents

1. Definition and relevance of biodiesel

Biodiesel is defined as the mono alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, for use in compression–ignition (diesel) engines. This specification is for pure (100%) biodiesel prior to use or blending with diesel fuel (US National Biodiesel Board, 2008). Its energy content and the physical and chemical properties are similar to conventional diesel fuel, allowing its use either on its own or mixed with conventional diesel in any diesel engine without requiring any modifications either to the ignition system or the fuel injector. Indeed, to date biodiesel is the only alternative fuel that can be used directly in existing engines. In addition, biodiesel possesses better lubricant properties which enhance engine yield and extend engine life (Vasudevan and Briggs, 2008). Biodiesel can be pumped, stored and handled using the same infrastructure, devices and procedure usually employed for conventional diesel fuel. In fact, as biodiesel does not

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produce explosive vapours and has a relatively high flash point (close to 150 °C), transportation, handling and storage are safer than with conventional diesel (Al-Zuhair, 2007).

In recent years there has been increasing interest in developing biodiesel as an alternative to fossil fuels, mainly in the automotive sector, because of its compatibility with current available engines. This interest is due to the following reasons:

(1) Environmental concerns related to climate change and the reduction of contaminant emissions. Among the advantages of using biodiesel we should note: (a) it is a quasi neutral fuel with respect to CO_2, since the only emissions are those previously fixed by photosynthesis; (b) it reduces the emission of the major atmospheric contaminants: particles in suspension, CO, polycyclic aromatic hydrocarbons, unburned hydrocarbons and especially sulphur, SO_x (Korbitz, 1999); (c) it is highly biodegradable and only slightly toxic whether in soils or aquatic environments (Lapinskiené et al., 2006); (d) biodiesel can be produced by recycling waste vegetable oils (i.e. frying–kitchen oils or refinery processed oils) allowing a valorisation of these residues.
(2) The rise in fossil fuel prices. According to calculations, locally produced biofuels are not feasible without any subsidy. However, steadily rising oil prices over USA $80/barrel suggest that biofuels may be economically viable in the medium term (Henniges and Zeddies, 2006); nowadays prices are around $100/barrel. In addition, other facts that may favour their feasibility are: (a) tax rules based on an international agreement for environmental protection. Tax rules include differential rates depending on the oilseed used, where they are grown, and whether they are produced by large agribusiness or family farmers. The regulatory frameworks deal with biodiesel–diesel percentage blends, forms of use and taxation (e.g. EU countries can adapt into national law the authorization leeway provided in EU Energy Tax Directive 2003/96 EC); (b) the optimization and genetic improvement of certain oleaginous plants (Akoh et al., 2007), or microalgae with high lipid contents (Xu et al., 2006; Li et al., 2007; Haag, 2007; Chisti, 2007, 2008); (c) the development of more efficient processes in the production, the recovery and purification of biodiesel, especially those based on *green* alternatives (i.e. substrates and catalysts fermented from renewable sources, making the biodiesel production fully environmental friendly) such as enzymatic biodiesel.
(3) The diversification of energy sources to guarantee supply, especially in the European countries that barely dispose of fossil fuel sources. The renewable features of biodiesel mean that it can be produced indefinitely, while current fossil fuel reserves will be exhausted in decades at current consumption rates (Vasudevan and Briggs, 2008).
(4) The need for a market for agricultural production surpluses (soybean in the U.S, rapeseed oil or sunflower oils in EU). Biofuels create added value and provide employment in rural areas (Henniges and Zeddies, 2006).

2. Starting oils for biodiesel production

The main raw materials used to produce biodiesel are the vegetable oils extracted from oleaginous plants. The cost of these materials currently represent about 70% of the total production costs (Behzadi and Farid, 2007); this means that the most suitable vegetable oils are those from crops with the highest productivity per hectare (Table 1) or low cost oils such as waste oils (Fairless, 2007). To produce biodiesel it is not necessary to refine crude oil completely. As a result, several steps involved in the refining process of edible oils (for example, deodorization, bleaching, etc) can be avoided.

Table 1
Oil yield (l/ha) from oleaginous species (Fairless, 2007) and microalgae.

Vegetable	Oil yield (l/ha)
Palm	2400
Jatropha	1300
Rapeseed	1100
Sunflower	690
Soybean	400
Microalgae[a]	18750
Microalgae[b]	58760

[a] *Phaeodactylum triconutum*, 20% oil (by wt.) in biomass, 5 g lipids/(m^2·day) (Acien Fernandez et al., 1998).
[b] 30% oil (by wt.) in biomass (Chisti, 2007).

The only necessary treatment is neutralization in the case of an alkaline catalytic process, or degumming in the case of an enzymatic transformation (Watanabe et al., 2002).

Nowadays, high petroleum-based sourced fuel prices, the collapse of food for biodiesel initiatives and concerns about increased levels of CO_2 emissions in the atmosphere have all created awareness of the need for alternative fuel solutions. Microalgae have optimistically emerged as one of the lowest cost feedstocks for biodiesel production. However, the production cost of high grade algae oils is, and likely will be, an obstacle in the short term. The main reason is that the operational conditions leading to high grade oil in microalgae are usually those providing low growth rate (low temperature, low light intensity and nitrogen deficiency) (Molina Grima et al., 1999). At present, it is rather difficult to get algae biomass with 20% lipid content below €1/kg, and even the recent advances in photobioreactors (closed and open) put algae oil costs below €5/kg.

Fats and oils may be characterized according to their physical (density, viscosity, melting point, refractive index, etc) or chemical properties (acidity, iodine index, peroxide index, saponification index, etc); these parameters will influence on the biodiesel quality. For example, the iodine index is related to the grade of oil unsaturation and in general, biodiesel produced from high unsaturated fatty acids containing oils are less viscous, show greater *cloud point* (i.e. the temperature at which fuel becomes cloudy due to solidification) and *pour points* (i.e. temperature at which fuel stops flowing) which make this biodiesel more suitable for cold weather conditions. However, it is also prone to oxidation, has a lower *cetane index* (related to reaction efficiency within the engine) and lower combustion heats. In contrast, the biodiesel produced from oils with a high proportion in long chain fatty acids (>18C) have a higher *cetane index* and combustion heat, but also lower *cloud* and *pour points* and greater viscosity (Knothe, 2005). This means that the fatty acid profile of the oil influences on quality of the biodiesel produced (Demirbas, 2008a). Table 2 shows the fatty acid profile of some of the raw materials used in biodiesel production.

Nowadays, among the many vegetable oils available, those with a high content in oleic acid are the most suitable due to the greater stability of their alkyl esters and their better characteristics as fuels (Knothe, 2005). In any case, according to existing legislation in EU and the US, biodiesel will need to comply with existing standards: the ASTM Biodiesel Standard D6751 in US and Standard EN 14214 in EU (Table 3) (Canakci and Sanli, 2008).

3. Conventional production methods

The direct use of vegetable oils as biodiesel is possible only by blending them with conventional diesel fuel in a suitable ratio, but the direct usage of vegetable oils in diesel engines is not technically possible because of their: (i) high viscosity; (ii) low stability against oxidation (and the subsequent reactions of polymerization), and (iii) low volatility, which influences on the formation of a relatively high amount of ashes due to incomplete combustion. Therefore, vegetable oils must be processed so as to acquire the properties