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# Biodiesel production from sunflower oil using extracellular lipase as catalyst

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Biodiesel consists monoalkyl esters of long chain fatty acids. It is produced from vegetable oil or fats by transesterification with methanol using chemical / enzyme lipase. This process has, therefore, been widely utilized for biodiesel fuel production in a number of countries. The enzymatic process offers several advantages over the chemical routes. The handicap of increase in process cost because of the cost of the enzyme can be overcome by using efficient production process for enzyme and using reusable derivatives of enzymes, such as extracellular enzyme. In the present investigation, therefore, optimization of process parameters for high lipase production by the microbes *viz.*, *Mucor racemosus, Rhizopus nigricans* and *Aspergillus terreus* using Czapek' Dox medium were carried out. Culture filtrates were used as extracellular crude enzyme source, as catalyst for conversion of sunflower oil to biodiesel and the parameters such as quantity of enzyme and methanol needed for significant yield of biodiesel was standardized.

Key words : Biodiesel, Lipase, Sunflower oil, Extracellular enzymes and Transesterification

## INTRODUCTION

The high energy demand in the industrialised world, as much in the domestic sector, as in transport and industry, its increase, and the derived problems of the widespread use of fossil fuels, make increasingly necessary the development of renewable energy sources of limitless duration and smaller environmental impact than the traditional ones.

The need for clean energy source is necessary because of the carcinogenic particulate emissions from diesel engines, which cause pollution and 'global warming'. The depleting reserves of petroleum – based products have also made scientists look for renewable sources of energy.

The upgrading of plant oils is a subject of great interest from an economic and societal point of view.

Energy crops have been considered as one of the best alternatives in the agricultural sector, whose production satisfies both food purposes and helps in the development of new industries such as the agro-energy industry. The concept of biodiesel addresses these twin issues.

Biodiesel obtained from energy crops produces favourable effects on the environment, such as a decrease in acid rain and in the greenhouse effect caused by combustion. Due to these factors and to its biodegradability, the production of biodiesel is considered an advantage to that of fossil fuels. In addition to this, it also shows a decrease in the emission of $CO_2$, $SO_2$ and unburned hydrocarbons during the combustion process.

"Biodiesel" means a monoalkyl esters that is derived from domestically produced vegetable oils, renewable lipids, rendered animal fats or any combination of those ingredients and meets the requirements of ASTM standards.

Vegetable oils are chemically triglycerides molecules, in which three fatty acids groups are esters attached to one glycerol molecule. The problem with direct use of such oils arise because of their higher viscosity and lower ignition quality as compared to diesel. The problems are more severe in the case of direct – injection engines than in the less efficient engines having precombustion engines. In the case of direct engines, very dilute blends of oils in diesel can be used. The production of free fatty acid methyl esters from the vegetable oils is a far more satisfactory approach. This conversion of the oil into the esters which are essentially a transesterification reaction, which can be catalysed by acid, alkali or using lipase enzyme.

Enzymatic transesterification is a potential method for modification of the physical and chemical properties of edible oils and fats. Oleic acid, present in large quantities in plant oils, is of special interest due to its high thermal-oxidation stability. New hybrid varieties of sunflower contain more than 80–90% of this fatty acid, whereas classic sunflower oil contains only 40% (Purdy, 1986).

Enzyme technology will be able to compete with the chemical route if selective and stable processes are developed. Selectivity will allow a precise formulation to be obtained according to the product desired. Stability of the process will significantly decrease the proportion of the enzyme price in the production cost of the product (Dossat *et al.*, 2002).

Chemical processes give high conversion of triacylglycerols (TAG) to their corresponding methyl esters (ME) but have drawbacks such as being energy intensive, difficulty in removing the glycerol, and the need for removal of alkaline catalyst from the product and treatment of alkaline wastewater.

Enzymatic methods can overcome these problems but have not been industrialized because of the high price and the instability of enzymes (Nelson *et al.*, 1996; Shimada *et al.*, 2002; Watanabe *et al.*, 2000).

This paper deals with preparation of lipase catalysed methyl esters from sunflower oil and its properties to ascertain its suitability as biodiesel.

## MATERIALS AND METHODS

The lipase catalyzed transesterification process was performed using extracellular lipase as catalyst. Extracellular lipase (Culture filtrates) produced by the microorganisms *Mucor racemosus*, *Rhizopus nigricans* and *Aspergillus terreus* in the Czapek's Dox medium using sunflower oil as carbon source was used.

The medium was standardized by modifying its original composition by varying the pH (4 to 9) temperature ($20^0$C to $40^0$C), nitrogenous sources $(NH_4)_2HPO_4$ (Di-ammonium hydrogen ortho phosphate) $(NH_4)_2SO_4$ (Di-ammonium sulphate), $NH_4Cl$ (Ammonium chloride) and $KNO_3$ (Potassium nitrate) and carbon sources (Glucose, sucrose, maltose and lactose along with sunflower oil).

***Transesterification process and optimization of process parameters: (Mahadik et al., 2002)***

Transesterification of sunflower oil to obtain biodiesel consists in replacing the glycerol of triglycerides with a short chain alcohol in the presence of a catalyst. For this purpose 100 ml of oil was taken and heated for 15 minutes and cooled. 10, 20, 30, 40 and 50 ml of culture filtrate (Source of crude enzyme) was taken and to this 20 ml of methanol was added and the content was stirred well. Then, it was poured into the oil and stirred for 1 hour. Mixture was heated to $40^0$C without stirring for 1 hour and it was poured in separating funnel which resulted in the formation of two layers. The top layer was methyl ester and bottom layer consisted of glycerol.

Since the ratio of alcohol to vegetable oil is one of the important factors that affect the yield as well as production cost of biodiesel, the quantity of methanol was varied from 20, 25, 30, 35, 40 and 45ml per 100ml of sunflower oil used with optimum quantity of catalyst.

***Separation and purification of biodiesel:***

After the completion of the reaction, the product was kept overnight for separation of biodiesel and glycerol layer. The catalysts and unused alcohol were present in the lower glycerol layer whereas a small quantity of catalysts, and alcohol were in the upper biodiesel layer. The upper esterified layer was collected and washed with hot distilled water and dried to remove traces of moisture.

***Physico-chemical properties of FAME:***

The fuel properties namely, density, kinematic viscosity, flash point, fire point, cloud point, pour point (Jain and Jain, 2006) acid value, iodine value, saponification value (Sadasivam and Manickam,1991) of sunflower oil biodiesel were determined as per the prescribed methods and compared with that of the conventional diesel as per American standards (ASTM, 2003) Acid value, iodine value, saponification value, (Sadasivam and Manickam,1991) of sunflower oil were also tested.

## RESULTS AND DISCUSSION

The fungi *Mucor racemosus* and *Rhizopus nigricans* could produce appreciable amount of lipase by just adjusting the pH of Czapek's Dox medium to pH 8 and *Aspergillus terreus* at neutral pH with oil as substrate at normal laboratory conditions. Further experiments were, therefore, carried out using these formulation and lipase thus, obtained was used for biodiesel production.

The process parameters such as quantity of methanol and extracellular enzyme (culture filtrate) when optimized suggested that 40ml of culture filtrate of *Mucor racemosus* and 30ml culture filtrates of either *Aspergillus terreus* and *Rhizopus nigricans* and 20 ml of methanol per 100ml of sunflower oil when used could yield maximum biodiesel (upto 80%). Irrespective of the quantity, these culture filtrates showed the presence of nearly 2000 units of lipase suggesting that for conversion of 100ml of oil to biodiesel upto 80%, 2000 IU of lipase is necessary (Fig. 1 and 2).

The properties of sunflower oil in terms of its acid value, iodine value, saponification value, density is presented in Table 1.The fuel properties of sunflower oil methyl esters were determined experimentally to ascertain their suitability as diesel fuel by using standard methods.

The fuel properties of biodiesel in terms of total free fatty acids, iodine value, saponification value, kinematic

**Table 1 : Comparison of biofuel parameters with ASTM standards**

| Methods / parameters | pH | FFA | I.V. | S.V. | K.V. | S.G. | C.P. | P.P. | Fl.P. | F.P. |
|---|---|---|---|---|---|---|---|---|---|---|
| Oil | - | 0.1 | 132.26 | 158.40 | | 0.91 | - | - | - | - |
| Diesel | - | - | 13.5 | 23.68 | 12.62 | 0.82 | - | - | 73$^0$C | 81$^0$C |
| M.R. | 7 | 0.05 | 64.84 | 180.61 | 15.65 | 0.86 | 2$^0$C | -6$^0$C | 175$^0$C | 181$^0$C |
| R.N. | 7 | 0.05 | 70.12 | 185.60 | 18.57 | 0.87 | -1$^0$C | -7$^0$C | 172$^0$C | 180$^0$C |
| A.T. | 7 | 0.05 | 63.9 | 178.50 | 16.95 | 0.87 | 1$^0$C | -6$^0$ C | 176$^0$C | 179$^0$C |
| American society for testing materials (ASTM) standards | Below 7 | 0.5 max | < 125.5 | 180 | 4.6 to 5 | 0.82 to 0.88 | -3$^0$C to 12$^0$C | - 15$^0$C to 10$^0$C | 130$^0$C Min | - |

FFA - Free fatty acids; I.V - Iodine value; S.V - Saponification value
K.V - Kinematic viscosity; S.G - Specific gravity; C.P - Cloud point
P.P - Pour point; Fl.P - Flash point; F.P - Fire point
M.R - *Mucor racemosus*; A.T - *Aspergillus terreus*; R.N - *Rhizopus nigricans*

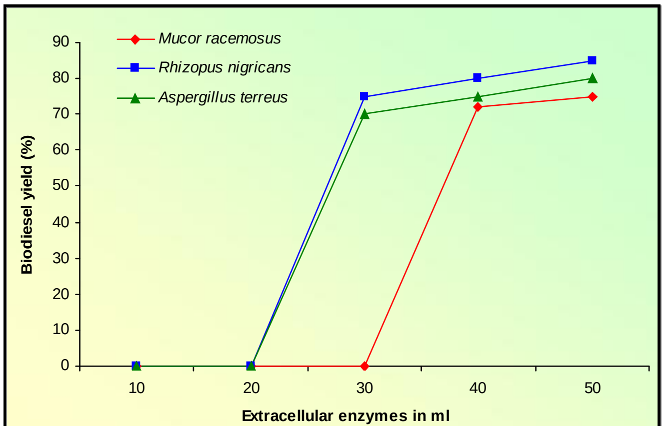


**Fig. 1 : Optimization of enzyme requirement**

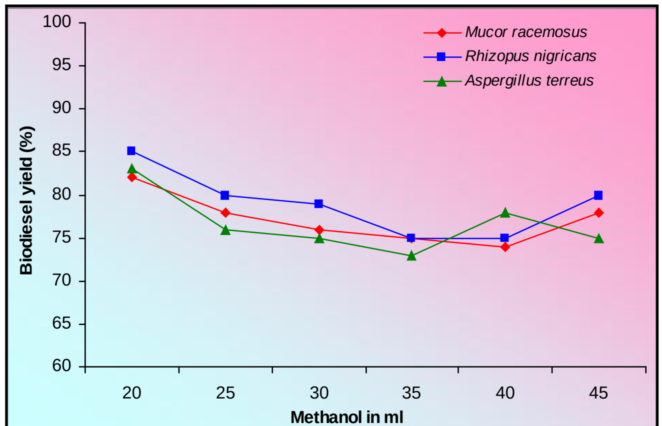


**Fig. 2 : Optimization of methanol**

viscosity, specific gravity, cloud point, pour point, flash point, fire point, and pH were tested and the results were compared with commercial diesel with ASTM standards.

Iodine value of biodiesel was higher than diesel but the values were within ASTM (American standard for testing methods)specifications. Saponification value of biodiesel was little higher than standards. Kinematic viscosity of biodiesel obtained seemed to be higher than diesel as well as ASTM standards. Specific gravity, cloud point and pour point were at par with diesel and ASTM standards. Flash point and Fire points were higher than diesel and ASTM standards for all the three biodiesel obtained using extracellular enzymes of all the three fungi studied.

From the results shown in Table 1, the viscosity of sunflower oil used was decreased by using transesterification methods. This is in agreement with the theory of Pischinger *et al.*(1982) that transesterification decreases the viscosities of vegetables oils, thus, enhancing their fluidity in diesel engines.

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