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Full Length Research Paper

Transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*

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Transesterification of vegetable oils is an important reaction that produces fatty acid alkyl esters, methyl and ethyl esters which are excellent substitutes for diesel fuel. Biodiesel prepared by catalyzed mild transesterification has become of much current interest for alternative fuel production. In the present study the ability of a commercial immobilized *Pseudomonas fluorescens* MTCC 103 to catalyze the transesterification of Jatropha oil and methanol was investigated. The cell of *P. fluorescens* was easily immobilized within the sodium alginate during batch process. The important parameters like reaction temperature, pH, oil/methanol molar ratio, amount of beads and reaction time was studied. From the study it was found that maximum yield of biodiesel was obtained at the optimum conditions of at 40ºC, pH of 7.0, molar ratio of 1:4, amount of beads of 3 g and reaction time of 48 h. The physical properties of the products were analyzed and the results were compared with conventional petroleum based diesel and it was found that the product can be used as an effective alternate fuel in existing diesel engine without any hardware engine modifications.

Key words: Transesterification, Biodiesel, Jatropha oil, *Pseudomonas fluorescens*.

INTRODUCTION

For more than two centuries, the world's energy supply has relied heavily on non-renewable crude oil derived (fossil) liquid fuels. Out of which 90% is estimated as to be consumed for energy generation and transportation. It is also known that emissions from the combustion of these fuels such as CO_2, CO, NO_X and sulfur containing residues are the principal causes of global warming. On the other hand, known crude oil reserves could be depleted in less than 50 years at the present rate of consumption. Thus, increased environmental concerns, tougher clean air act standards necessitates the search for a viable alternative fuels, which are environment friendly. Oil seed crops such as palm soyabean, sunflower, peanut, olive, etc., are by far the largest group of exploitable renewable biomass resource for liquid fuel and energy generation (An-Fei and Kerby, 2002). The attractive features of bio-diesel fuel are: (i) it is plant – derived, not petroleum – derived, and as such its combustion does not increase current net atmospheric levels of CO_2, a "greenhouse" gas; (ii) it can be domestically produced, offering the possibility of reducing petroleum imports; (iii) it is biodegradable; (iv) relative to conventional diesel fuel, its combustion products have reduced levels of particulates, carbon monoxide, sulfur oxides, hydrocarbons, soot, and under some conditions, nitrogen oxides (Antolin and Tinaut, 2002). Vegetable oils can be used in diesel engines as they have a high octane number and calorific value very close to diesel.

Transesterification of vegetable oils is an important reaction that produces fatty acid alkyl esters that are valuable intermediates in oleo chemistry, and methyl and ethyl esters which are excellent substitutes for diesel fuel. Transesterification as an industrial process is usually carried out by heating an excess of the alcohol with vegetable oils under different reaction conditions in the presence of an inorganic catalyst. The most commonly used catalysts are alkali hydroxides and alcoholates. Transesterification is also possible under acidic conditions, but this process requires higher reaction temperatures.

Chemical methanolysis using an alkali catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the

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reaction has several drawbacks: it is energy intensive; recovery of glycerol is difficult, the alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment, and free fatty acids and water interfere with the reaction. Recently, enzymatic methanolysis using lipase has become more attractive for biodiesel fuel production, since it can overcome the problems mentioned above. In particular, it should be noted that the byproduct glycerol, can be easily recovered without complex processing and also that free fatty acids contained in waste oils and fats can be completely converted to methyl esters (Mamoru et al., 2001; Oznur and Melek, 2002). Effective methanolysis reactions using several lipases from *Candida* species, *Pseudomonas* species and *Rhizopus* species have been developed by several researchers. With these lipases, methyl ester content in the reaction mixture of more than 90% is obtained using either low or high water content systems. However, the use of extracelluar lipase as a catalyst requires complicated recovery, purification and immobilization processes for industrial application (Bank et al., 2001).

Consequently, there has been considerable interest in the direct use as a whole cell biocatalyst of intracellular lipases (Kaieda et al., 1999; Matsumoto et al., 2001). For the industrial interesterification of fats and oils, *Pseudomonas* species immobilized with sodium alginate gel can be used directly as a whole cell biocatalyst. In the present study *Pseudomonas fluroscence* cells immobilized within sodium alginate gel as a whole cell biocatalyst was utilized for biodiesel fuel production from jatropha oil (Foidl et al., 1996). Methanolysis reaction was carried out with addition of methanol in the presence of 10 – 20% water in a batch operation produced a methyl ester content of 70 – 75% in the reaction mixture without organic solvent pretreatment.

MATERIALS AND METHODS

Materials

The non-edible crude Jatropha oil was purchased commercially and was stored at 4°C to avoid rancidity of the vegetable oil. Its quality characteristics were determined according to the standard methods of fats and oils published by the association of oil chemists, which has the density of 0.92 g/cm^3, acid value of 19.635 mg KOH, saponification value of 187 gm KOH and free fatty acid of 17.25 mg KOH/g and it was used through out the experimentation.

P. fluorescens, MTCC 103 was obtained from Microbial Type Culture Collection and Gene Bank, Chandigargh (India). The culture was maintained on nutrient agar medium. After three days incubation at 25°C the agar slants were stored at 4°C. The liquid medium for the growth of inoculum for bacteria was nutrient agar medium composed of 1.0 g/l of beef extract, 2.0 g/l of yeast extract 5.0 g/l of peptone and 5.0 g/l of NaCl.

Inocula preparation

Inocula were grown aerobically in 250 ml Erlenmeyer flasks containing the above mentioned medium at 25°C in an Environmental Shaker (Remi Scientific) at 200 rpm for 24 h. Active cells were centrifuged in a clinical centrifuge (1200 rpm), washed with sterile water, and were used as inoculum.

Immobilization of *Pseudomonas fluorescence* cells by entrapment

The sodium alginate entrapment of cells was performed according to the standard method. Alginate solution with a concentration range of 0.5 - 10% was used for the cell immobilization and was prepared by dissolving sodium alginate in boiling water and autoclaved at 121ºC for 15 min. Both alginate slurry and cell suspension was mixed and stirred for 10 min to get a uniform mixture the alginate/ cell mixture which was extruded drop by drop into a cold sterile 0.2 m $CaCl_2$ solution through a sterile 5 ml pipette from 5 cm height and kept for curing at 4ºC for 1 h. The beads were hardened by resuspending into a fresh $CaCl_2$ solution for 24 h at 4ºC with gentle agitation. Finally these beads were washed with distilled water to remove excess calcium ions and unentraped cells. When the beads are not being used, they are preserved in 0.9% sodium chloride solutions in the refrigerator.

Methanolysis of Jatropha oil

Methanolysis reactions were conducted at stoichiometric molar ratio of oil/methanol; oil and methanol were poured into the reaction flask and heated to the reaction temperature with constant shaking using reciprocal shaker (150 osicillations/min; amplitude 70 mm) for 48 h. In subsequent experiments, in which the effect of molar ratio of oil/ methanol was investigated, the volume of oil is kept constant and the volume of methanol is varied. Around 3 ml of hexane is added to the reaction mixture to increase the solubility of the reactants (Mohamed and Uwe, 2003). The appropriate amount of immobilized whole cells based on oil weight was added to the flask. After 48 h of reaction time, the reaction was stopped and the cells were removed from the reaction mixture by filtration. The produced ester and byproduct glycerol were separated using separate funnel. Qualitative analysis of ester product was carried out by column chromatography (Yong and Shiyi, 2007). Esterification reactions were carried out in duplicate and results were shown graphically.

RESULTS AND DISSCUSION

Effect of temperature

Temperature is one of the important parameter for the production of biodiesel because the rate of reaction is strongly influenced by the reaction temperature. The effect of temperature on biodiesel production from Jatropha oil using immobilized cells of *P. fluorescens* was studied by conducting experiments at different temperatures 30, 35, 40, 45 and 50ºC keeping initial immobilized cell concentration of 3 g of beads, substrate concentration of 50 ml of oil (1:3 molar ratio of oil to methanol) with n-hexane (3 ml), reaction time of 48 h and pH of 7.0 were fixed constant. For each temperature the yield of biodiesel was found and is shown in Figure 1. Maximum yield of 71% of biodiesel was obtained at temperature 40ºC. Figure 1 show that during the course of transesterification the biodiesel yield continues to increase uniformly till 40°C and was found to be decreasing after 40°C due to denaturation of the enzyme. The optimum temperature for the maximum yield of biodiesel was fixed as 40°C.

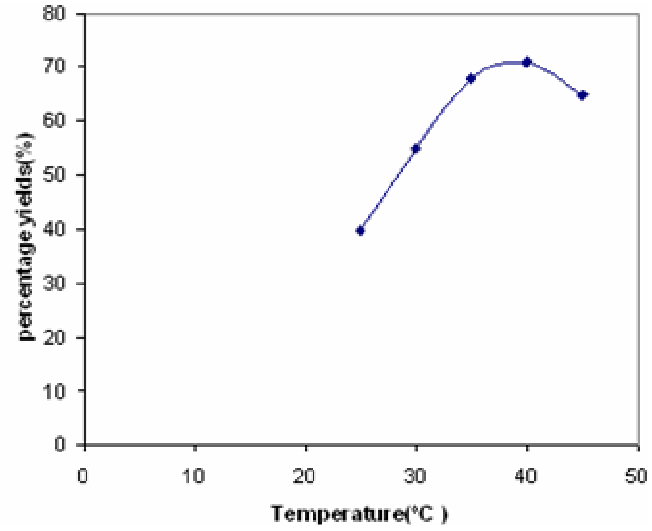

Figure 1. Effect of temperature on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

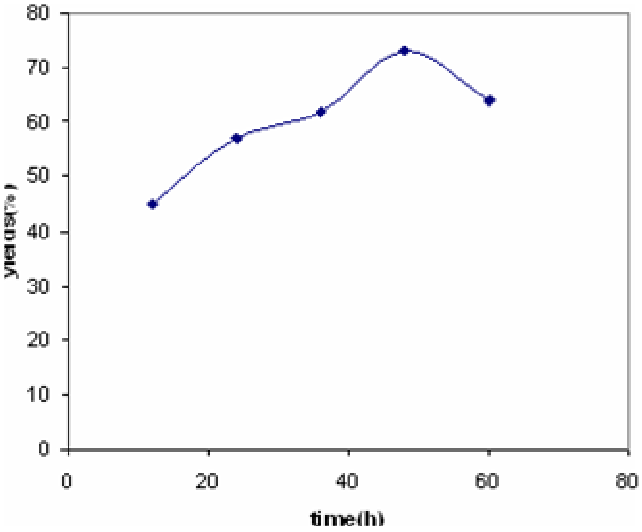

Figure 3. Effect of reaction time on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

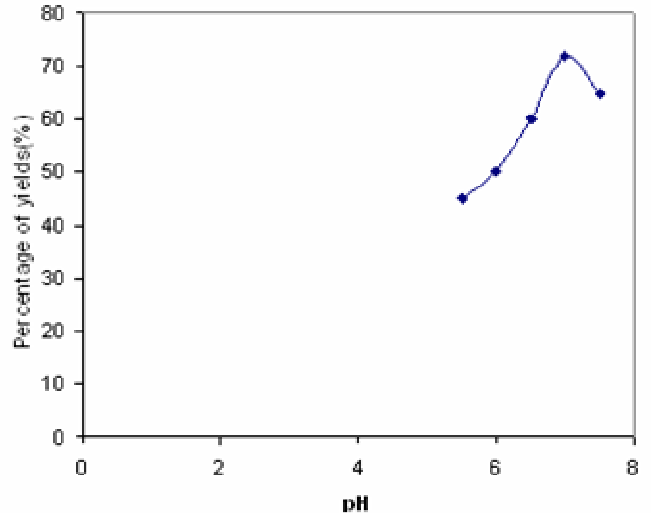

Figure 2. Effect of pH on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

Effect of pH

pH of the reaction media is the another important variable affecting the yield of biodiesel. Depending on the pH the amount of ester produced will be varying. In this experimental study, the pH of reaction media was varied from 5.5 to 7.5. Effect of pH on biodiesel production from Jatropha oil using immobilized cells of *P. fluorescens* was studied by conducting experiments with different pH viz 5.5, 6.0, 6.5, 7.0 and 7.5. Experiments were carried out for the reaction period of 48 h at an optimum temperature of 40ºC, immobilized cell concentration of 3 g beads and substrate concentration of 50 ml of oil (1:3 molar ratio of oil to methanol) with n-hexane (3 ml). From Figure 2 the yield of biodiesel was found to be increasing and a maximum (72%) was obtained at pH 7.0 and the yield of bio diesel was found to be decreasing when the pH was increased beyond 7.0.

Effect of reaction time

Effect of time on biodiesel production from Jatropha oil using immobilized cells of *P. fluorescens* was studied by conducting experiments with different periods of 12, 24, 36, 48 and 60 h. Experiments were carried out at the optimum temperature 40ºC, pH 7.0, immobilized cell concentration of 3 g beads and substrate concentration of 50 ml of oil (1:3 molar ratio of oil to methanol) with n-hexane (3 ml) as constant. Figure 3 shows that on increasing the reaction time the percentage yield of biodiesel increases till 48 h and thereafter decreases. Further increase in the reaction time does not have effect on the production of biodiesel.

Effect of amount of beads

The effect of amount of immobilized beads on production of biodiesel from jatropha oil using immobilized cells of *P. fluorescens* was studied by conducting experiments at different amounts viz 1, 2, 3, 4 and 5 g at constant levels of substrate concentration of 50 ml of oil (1:3 molar ratio of oil to methanol) and n-hexane (3 ml) at optimum temperature 40ºC, pH 7.0 and reaction time 48 h. Figure 4 shows that on increasing the amount of beads the percentage of yield of biodiesel increases till 4 g (70%)

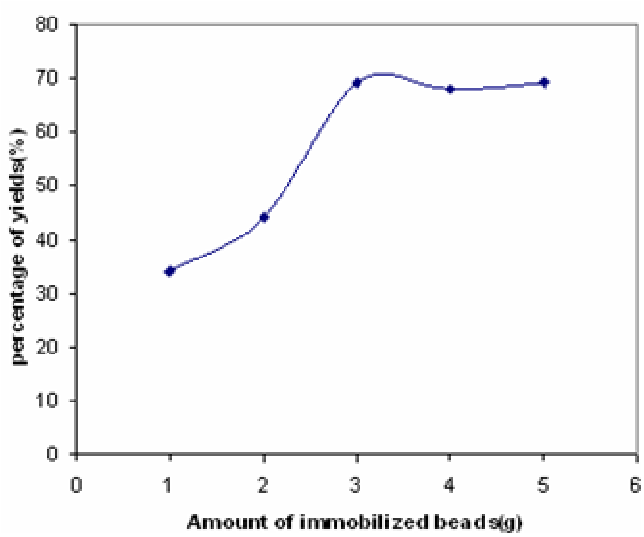

Figure 4. Effect of amount of catalyst on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

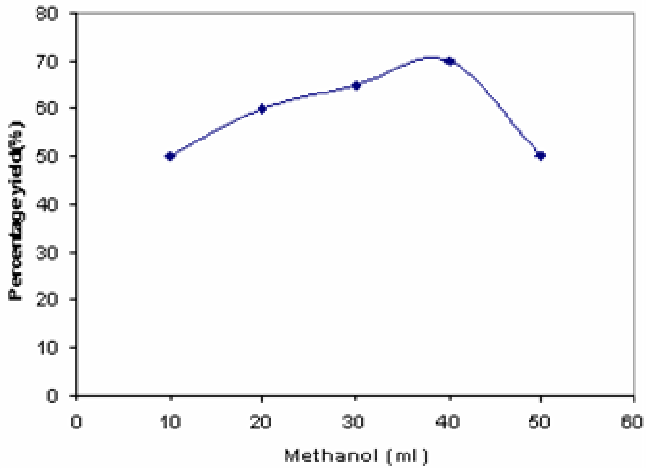

Figure 5. Effect of oil/ alcohol molar ratio on biodiesel yield during transesterification of Jatropha oil using immobilized *Pseudomonas fluorescens*.

and there after decreases, so 4 g was chosen as the optimum amount of beads.

Effect of oil/ alcohol molar ratio

Another important variable affecting the yield of biodiesel is the molar ratio of oil to alcohol. Because to shift the transesterification reaction in forward direction, it is necessary to use either an excess amount of alcohol or to remove one of the products from the reaction mixture. Effect of molar ratio on biodiesel production from Jatropha oil using immobilized cells of *P. fluorescens* was studied by conducting experiments with different ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 (oil to methanol). Experiments were carried out for the reaction periods of 48 h at an optimum temperature of 40ºC, immobilized cell concentration of 3 g beads and pH 7.0. From Figure 5 by increasing the molar ratio, the yield of bio diesel was found to be increasing and a maximum yield of 70% was obtained at molar ratio of 1:4. The yield of biodiesel was found to be decreasing when the molar ratio was increased beyond 1:4. It may be due to the inhibition of excess methanol reduces the enzyme activity.

Conclusion

Transesterification reaction was carried out using Jatropha oil and short chain alcohol (methanol on hexane) using immobilized cells of *P. fluorescens* MTCC 103. The various parameters affecting biodiesel yield namely temperature, pH, reaction time, amount of beads and molar ratio of oil to alcohol were studied. The maximum yield of 72% was obtained at optimum values of temperature 40ºC, pH 7, reaction time 48 h, amount of beads 4 g and molar ratio of oil to alcohol 1:4. The biodiesel produced was analyzed for its physical properties and compared with the values of petroleum based diesel. The specific gravity, flash and fire point, cloud and pour point, kinematic viscosity are slightly lower than that of diesel, whereas the diesel index is much higher and the smoke point is slightly lower.

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