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Transesterification of used vegetable oil using immobilized lipase enzyme

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ABSTRACT

Lipase immobilized on Santa Barbara Acid-15 (SBA 15) produced from economic precursor obtained from sugarcane leaf ash was used as a catalyst for methanolysis of used vegetable oil. Here, highly ordered mesoporous silica has been prepared from sugarcane leaf ash by template assisted method. BET surface area, primary pore size and pore volume of SBA – 15 were 587 m²/g, 7 nm and 0.8 cm³/g respectively. Mesoporous silica had shown percentage immobilization of 48.92% and specific enzyme activity of 8.6 U/ mg. The optimal conditions for processing 5 g of used vegetable oil are: 100 mg of immobilized lipase, 8:1 molar methanol to oil ratio and a temperature of 40 °C. 96.5 % yield of fatty acid methyl ester (FAME) was obtained under optimized conditions.

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KEYWORDS

Biodiesel;
SBA-15;
Methanolysis;
Lipase immobilization;
Used vegetable oil.

INTRODUCTION

Fossil fuels which serve as the prime source of energy are expected to completely deplete in the near future since the rate at which they are formed is negligible compared to the rate at which they are consumed. Moreover the atmospheric emissions produced by burning of fossil fuels have contributed to about 90 percent of the earth's greenhouse gases^[1]. This has generated the need for a clean renewable fuel that could serve as a better replacement for the fossil based fuels and at the same time prolong their availability by decreasing their consumption. Biodiesel can be defined as a fatty acid methyl ester (FAME) when produced by methanolysis^[2]. Biodiesel has to be produced only from used vegetable oil, preferably available in abundant so as to minimize the food-versus-fuel debate^[3]. More-

over, pretreatment steps involved in chemical methods add up to the cost of production. Enzymatic methanolysis can be used as an effective alternate to the existing methods^[4]. Continuous operation, recovery and regeneration of the catalyst can be accomplished by immobilizing enzymes on inert support. Mesoporous silica as a credible support for enzyme immobilization has been studied^[5]. Used vegetable oil as a source for biodiesel production by lipase immobilization on an economic host material goes a long way in reducing the biodiesel production cost^[6].

Many methods were reported for SBA-15 synthesis using different precursors which are costly and to reduce the cost of the support, use of an economic precursor is a prerequisite. Although some work has been reported on converting ash into mesoporous silica materials, it is less explored. Sugarcane leaf ash has been

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used as a partial replacement of portland cement due to its low cost and high SiO₂ content. An alternative silica source obtained from sugarcane leaf ash is a promising option.

Our study focuses on the methanolysis of used vegetable oil catalysed by lipase immobilised on mesoporous SBA-15 in a batch process, wherein the SBA-15 was prepared by condensation of sodium silicate, a low cost silica precursor obtained from sugarcane leaf ash. The optimum parameters such as temperature, methanol to oil molar ratio and reusability of lipase immobilized on the support matrix were studied.

MATERIALS AND METHODS

Materials

Synthesis of SBA-15 involves the use of sugarcane leaf ash as the silica precursor. Used vegetable oil was brought from a local restaurant. The chemical composition of the oil is given in TABLE 1. Pluronic P123 a triblock co-polymer, (99%, Aldrich) (PEO₂₀PPO₇₀PEO₂₀) was used as a template. Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Copper Sulphate Pentahydrate, Sodium Potassium Tartarate were purchased from Himedia Laboratories Pvt. Ltd. Lipase (97% pure) was purchased from Himedia Laboratories Pvt. Ltd.

TABLE 1 : Fatty acid composition of used vegetable oil (%)

Fatty acid	Molecular formula	wt %
Palmitic acid	C16:1	21.25
Stearic acid	C18:0	8.75
Oleic acid	C18:1	47.12
Linoleic acid	C18:2	18.34
Linolenic acid	C18:3	0.20
Myristic	C14:0	1.45
Arachidic	C20:0	0.47
Gadoleic	C20:1	0.12
Others		2.3

Preparation of mesoporous silica support and immobilization

Mesoporous SBA-15 was synthesized using template assisted method^[7]. Sugarcane leaves obtained from the nearby fields and were sun dried till they loose all the moisture. Leaves after drying, were reduced to small

sizes by cutting. The size reduction of leaves facilitates complete burning. Burning was carried out in a crucible furnace (650°C). 1 g of finely ground ash was then fused with sodium hydroxide in the ratio 1:1.2 by weight, at a temperature of 400 °C for 1 h. The dilution of fused mass with water in the ratio 1:4 by weight was done. It was followed by sonication to disperse the particles completely^[8]. Lipase (20 mg) and pH 7.5 phosphate buffers (10 ml) were added to 2 g of mesoporous material. The mixture was kept in a vial in a deep freezer for 24 h^[9,10].

Characterization

The reaction mixture containing methyl esters was analyzed by Gas chromatography – Mass spectroscopy (CLARUS 500, PerkinElmer, USA). Surface area, pore volume and pore diameter of the silica sample were determined using BET analysis.

Methanolysis

5 g of used vegetable oil (molecular weight 720 g/mol), 0.4 g of water and 0.88 g of methanol (alcohol: oil molar ratio 4:1), were mixed and a batch reaction was carried out in 10mL screw-capped vials. By adding 100 mg of immobilized lipase to the substrate mixture, the reaction was carried out at 30°C for 8h. Vials were incubated in a rotary water bath at 80 oscillations/min.

RESULTS AND DISCUSSION

Characterization

Immobilization of lipases was done on mesoporous material and the specific enzyme activity of the immobilized lipase was 8.6 LU/ mg^[11]. SBA-15 synthesized had the typical wheat-like morphology and high porous surface as reported earlier^[11]. The surface area was determined to be 573 m²g⁻¹. Average pore volume and pore diameter were found to be 0.8 cm³ g⁻¹ and 7 nm respectively. Presence of –OH and Si–O–Si bond in SBA-15 had been shown earlier in our previous reports^[12].

Effect of Methanol to oil ratio

Methanol to oil ratio is one of the important process variables affecting the yield of FAME. The results

are shown in the Figure 1. Methanol to oil ratio 4:1 showed maximum FAME yield. From the Figure 2 it can be seen that the percentage yield of FAME increased with increase in methanol to oil molar ratio and reaches a maximum. Increase in concentration of methanol removes the water layer over the lipase hence leads to enzyme deactivation. Beyond the certain ratio the biodiesel production yield decreased. This might be due to the resistance caused by the water layer to the diffusion of substrate into immobilized lipase^[13].

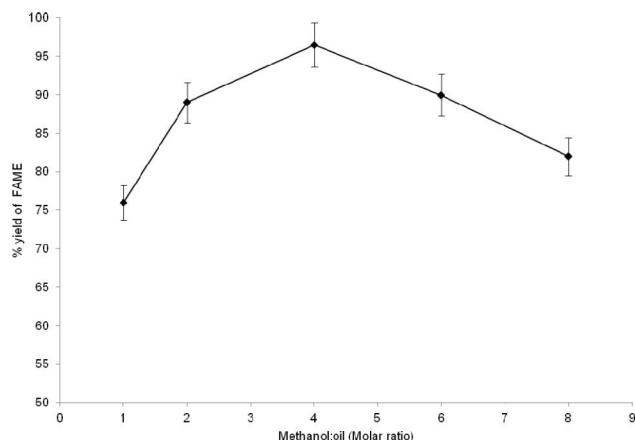


Figure 1 : Effect of Methanol to oil molar ratio on immobilized lipase catalyzed methanolysis of used vegetable oil for Temperature 40°C, 4% v/v water content and reaction time of 8h

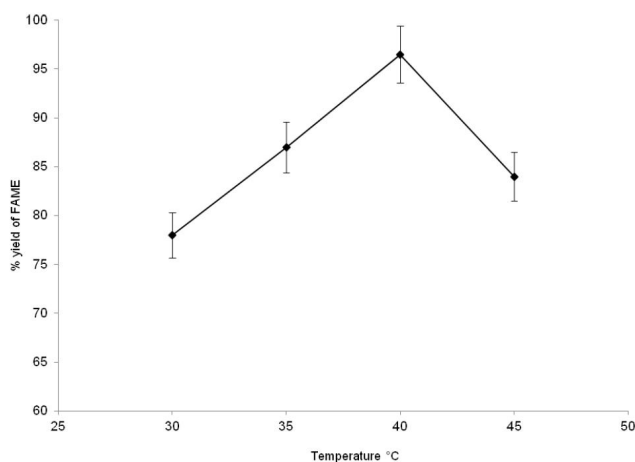


Figure 2 : Effect of temperature on immobilized lipase catalyzed methanolysis of used vegetable oil for 8: 1 molar ratio of methanol to oil, 4%v/v water content and reaction time of 8h.

Effect of temperature

For temperatures 30 °C, 35 °C, 40 °C and 45 °C methanolysis was carried out keeping other parameters fixed. The percentage yield of FAME was maximum at 40°C (Figure 2). With increase in temperature there

was significant increase in yield. It was mainly due to increase in enzyme activity with temperature. But at higher temperatures i.e. temperature more than 40 °C, the enzyme loses its activity resulting in low FAME yield^[14].

CONCLUSION

Mesoporous SBA-15 obtained from sugarcane leaf ash. SBA-15 prepared Sugarcane leaf ash showed a higher specific surface area and pore volume. The BET surface area has been determined to be 573 m²g⁻¹, pore diameter 7 nm and pore volume 0.84 cm³/g. The optimum parameters were studied and identified with methanol to oil molar ratio being 8:1, temperature 40°C and a high FAME yield of 96.5% was obtained.

REFERENCES

- [1] A.E.Atabani, A.S.Silitonga, Irfan Anjum Badruddin, T.M.I.Mahlia, H.H.Masjuki, S.Mekhilef; *Renew.Sustainable. Energy.Rev.*, **16**, 2070–2093 (2012).
- [2] Fei-ling Pua, Zhen Fang, Sarani Zakaria, Feng Guo and Chin-hua Chia, *Biotechnol.Biofuels.*, **4**, 56 (2011).
- [3] M.Mohamed, Soumanou, T.Uwe, Bornscheuer; *Enzyme.Microbial. Technol.*, **33**, 97–103 (2003).
- [4] Mirosawa Szczesna Antczak, Aneta Kubiak, Tadeusz Antczak, Stanislaw Bielecki; *Renew.Energy.*, **34**, 1185–1194 (2009).
- [5] N.W.Fadnavis, G.Sheelu, B.M.Kumar, M.U.Bhalerao, A.A.Deshpande ;*Biotechnol.Prog.*, **19**, 557–564 (2003).
- [6] Andrea Salis, Marcella Pinna, Maura Monduzzi, Vincenzo Solinas; *J.Mol.Catal.B: Enzym.*, **54**, 19–26 (2008).
- [7] Li Chen, Yi Meng Wang, Ming-Yuan He; *J.Porous.Mater.*, doi:10.1007/s10934-010-9372-6, (2010).
- [8] A.Arumugam, V.Ponnusami; *Indian J.Chem.Technol.*, **20**,101-105, (2013).
- [9] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; *J.Biol.Chem.*, **193**, 265-275 (1951).
- [10] E.Sharan, M.Y.Kamat; *Res.J.Chem.Enviro...*, **5**, 2 (2001).
- [11] A.Arumugam, V.Ponnusami; *Biomass Conv.Bioref.* DOI 10.1007/s13399-013-0087-1.

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- [12] A.Arumugam, V.Ponnusami; J.Sol-Gel. Sci.Technol., **67**, 244–250 (2013).
- [13] Mamoru Iso, Baoxue Chen, Masashi Eguchi, Takashi Kudo, Surekha Shrestha; J.Mol.Catal B: Enzym., **16**, 53–58 (2001).
- [14] N.Kaile, X.Feng, F.Wang, T.Tianwei; J.Mol.Catal.Enzym., **43**, 142-147 (2006).
- [15] Y.Chen, B.Xiao, J.Chang, Y.Fu, P.Lv, X.Wang; Energy.Convers. Management., **50**, 668-673 (2009).