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FULL LENGTH ARTICLE

Enzymatic biodiesel production from palm oil and palm kernel oil using free lipase

S.O. Kareem [a,*], E.I. Falokun [a], S.A. Balogun [a], O.A. Akinloye [b], S.O. Omeike [c]

[a] *Department of Microbiology, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria*
[b] *Department of Biochemistry, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria*
[c] *Institute for Human Resources Development, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria*

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Abstract Biodiesel from biological materials is receiving attention as alternative fuel. This investigation compared quality of biodiesel produced from lipase-transesterified palm oil (PO) and palm kernel oil (PKO) based on fatty acid methyl esters (FAME) and fuel properties. Biodiesel yield was optimized using three-level four-factor of Design Expert Software with enzyme load (2.5–7.5%), methanol-oil molar ratio (3-1, 1), and temperature (30–40 °C) as variables. Biodiesel properties FAME, Flash Point (FC), Pour Point (PP) and kinematic viscosity were compared with American (ASTM D6751) and European (EN 14214) Standards. PO (>90%) biodiesel yield was higher than PKO (<90%), both with maximum yields observed at 40 °C, 3:1 and 5–7.5%. FAME in PO-biodiesel (POBD) and PKO-biodiesel (PKOBD) include Hexadecanate and 9-Octadecenoate, while POBD had more unsaturated FAME (Dodecanoate). POBD and PKOBD had PP 6.7 °C and 17.7 °C respectively, while POBD Kinematic viscosity (813 kg/m^3) agreed with both standards. This study showed that POBD could be a better fuel alternative with further improvement of fuel properties.

1. Introduction

Energy is the most fundamental requirement for human existence and activities; however, non-renewable energy source that contributes over 86% of the global energy supply is depleting [1]. Shortage of resources and high crude oil prices have led to the search for a new alternative and renewable energy source such as biodiesel [2].

Biodiesel is renewable energy fuel consisting of long chain fatty acid derived from vegetable oils or animal fats and is non-toxic, biodegradable, renewable, does not contain sulfur and aromatic compounds, among other advantages [3,4]. Enzymatic conversion of oils to biodiesel by lipases as biocatalysts is receiving much interest in biodiesel production due to its high efficiency, selectivity and production of a highly purified product [5]. Oils from various feedstocks are used for the enzymatic production of biodiesel, with vegetable oil currently being used worldwide as a sustainable commercial feedstock [3]. Investigations have also been carried out into the use of other edible oils and feedstocks such as sunflower, soyabeans,

* Corresponding author.
E-mail address: sharafkareem@yahoo.co.uk (S.O. Kareem).

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rapeseed, corn etc. to supplement competition for vegetable oil with food producers which results in concerns of food security.

Palm oil and palm kernel oil are found in the flesh and seed (kernel) of the palm fruit respectively. They are two of the major crop found in Nigeria, being a tropical country with a wide variation of climatic and soil condition [6]. A 2008 report showed that palm oil and palm kernel rank first and third in the estimated list of annual production of major Nigerian vegetable oils, while the production size is also expected to increase [7]. This projection indicates the possibility of continued use of these oils in biodiesel production. This research investigation aimed at producing biodiesel from palm and palm kernel oil using free lipase.

2. Materials and methods

Lipase-producing strain of *Aspergillus niger* was obtained from the Culture Collection Center of Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria. Palm oil (PO) and palm kernel oil (PKO) were obtained locally from an oil palm industry. All chemicals used including tween 80, gum arabic, thymolphtalein, sodium dihydrogen phosphate, monosodium hydrogen phosphate, sodium potassium tartarate, copper sulfate pentahydrate were of analar grade.

2.1. Lipase production and assay

Lipase production by *A. niger* via solid state fermentation was done as previously described [8], while lipase activity was determined using a combined method [9,10]. Spores of the mold was inoculated in medium containing rice bran, palm kernel cake, groundnut cake and starch (5:5:3:1 w/w), moistened with distilled water to 55% and incubated at 30 °C for 72 h. The fermented moldy bran was mixed with 50 mM sodium phosphate buffer pH 8 (1:10w/v) and placed in an orbital shaker at 150 rpm at 28 °C for 2 h. The mixture was filtered using glass microfiber and the clear filtrate was used as crude lipase.

Olive oil substrate emulsion was prepared by mixing 25 ml of olive oil with 75 ml of 7% Arabic gum solution in a conical flask and incubate at 37 °C for 15 minutes using a water bath (Nickel Electro Ltd, England). Reaction mixture was made up of 50 ml olive oil emulsion and 10 ml crude enzyme incubated at 50 °C for 30 min with intermittent shaking in water bath. At 5-min intervals, 5 ml of reaction mixture was removed and mixed with 5 ml ethanol (95%) and thymolphtalein indicator (2–3 drops) in a conical flask to stop the reaction. The released fatty acid was titrated with sodium hydroxide (0.05 N) in a buret until a light blue color appears. Control experiment was done by mixing phosphate-buffered olive oil (5 ml) with

Table 1 Palm oil (PO) biodiesel yield (%) of different experimental runs.

Run	Factor 1 A:Temp (Celsius)	Factor 2 B:molar ratio (Molarity)	Factor 3 C:enzyme load (percent)	Factor 4 D:agitation (rpm)	Response biodiesel yield (percent)
1	35.00	2.00	5.00	220.71	94
2	30.00	3.00	2.50	100.00	94
3	30.00	1.00	2.50	100.00	90
4	30.00	1.00	7.50	100.00	90
5	35.00	2.00	5.00	150.00	94
6	35.00	2.00	5.00	150.00	94
7	40.00	3.00	7.50	200.00	95
8	35.00	2.00	5.00	150.00	94
9	40.00	1.00	2.50	200.00	93
10	40.00	3.00	2.50	100.00	94
11	35.00	0.59	5.00	150.00	90
12	35.00	2.00	5.00	150.00	94
13	30.00	3.00	2.50	200.00	94
14	40.00	1.00	7.50	200.00	93
15	40.00	1.00	2.50	100.00	93
16	35.00	2.00	5.00	79.29	94
17	35.00	3.41	5.00	150.00	95
18	30.00	3.00	7.50	200.00	93
19	40.00	3.00	7.50	100.00	95
20	40.00	1.00	7.50	100.00	90
21	35.00	2.00	5.00	150.00	94
22	30.00	1.00	2.50	200.00	89
23	40.00	3.00	2.50	200.00	94
24	27.93	2.00	5.00	150.00	93
25	35.00	2.00	5.00	150.00	94
26	35.00	2.00	1.46	150.00	93
27	30.00	3.00	7.50	100.00	93
28	30.00	1.00	7.50	200.00	90
29	35.00	2.00	8.54	150.00	92
30	42.07	2.00	5.00	150.00	95

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