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Biodiesel production by lipase-catalyzed transesterification of *Ocimum basilicum* L. (sweet basil) seed oil

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ABSTRACT

The increasing global demand for fuel, limited fossil fuel resources, and increasing concern about the upturn in gaseous CO_2 emissions are the key drivers of research and development into sources of renewable liquid transport fuels, such as biodiesel. In the present work, we demonstrate biodiesel production from *Ocimum basilicum* (sweet basil) seed oil by lipase-catalyzed transesterification. Sweet basil seeds contain 22% oil on a dry weight basis. Artificial neural network with genetic algorithm modelling was used to optimize reaction. Temperature, catalyst concentration, time, and methanol to oil molar ratio were the input factors in the optimization study, while fatty acid methyl ester (FAME) yield was the key model output. FAME composition was determined by gas chromatography mass spectrometry. The optimized transesterification process resulted in a 94.58% FAME yield after reaction at 47 °C for 68 h in the presence of 6% w/w catalyst and a methanol to oil ratio of 10:1. The viscosity, density, calorific value, pour point, and cloud point of the biodiesel derived from sweet basil seed oil conformed to the EN 14214 and ASTM D6751 standard specifications. The antioxidant stability of the biodiesel did not meet these specifications but could be improved via the addition of antioxidant.

1. Introduction

Solar, wind, and biomass energy are alternatives to fossil fuels and are becoming well-established as commercial sources of renewable energy [1]. Biofuels are carbon compounds produced from biomass that can directly substitute for, or be used as blends with, petroleum-derived liquid transportation fuels [2]. Further, elevated and increasing atmospheric CO_2 levels is driving increased demand for alternative, sustainable transportation fuels. Biodiesel, which is produced from the reaction between triglycerides and short chain alcohols, can be used to power existing diesel engines without modification and is renewable, non-flammable, biodegradable, and non-toxic. Further, biodiesel has additional benefits compared with petroleum-derived diesel, including a reduction in engine emission of particulates, NO_x, SO_x, CO and hydrocarbons, reduced toxicity, improved safety, and lower lifecycle of CO_2 emissions [3].

Biodiesel production from edible oils raises the concerns of long-term competition between feedstocks for biofuel production and the supply of food [4], and hence the use of inedible oils for biodiesel production is growing worldwide [5]. To date, several inedible oils have been investigated as feedstocks for biodiesel production including seed oils from *Jatropha curcas* (Jatropha), *Calophyllum inophyllum* (Indian laurel), tobacco, rubber, mahua, cotton seed, karanja, castor, and *Schleichera oleosa* (Kusum). Of these feedstocks, Jatropha seed is the most widely accepted as a source of inedible oil with significant potential for biodiesel production; however, it only grows well in tropical and sub-tropical environments [6]. The protein-rich residue left over after oil extraction from Jatropha seed is a potential ingredient in animal feed but it cannot be used directly without detoxification [7]. Accidental consumption of the seeds by humans have been reported to cause symptoms including giddiness, vomiting, and diarrhoea and, in extreme cases, consumption can be fatal [8]. Therefore, we introduce a novel feedstock for biodiesel production, *Ocimum basilicum* L (sweet basil), which is widely available in the tropics and subtropics.

O. basilicum L. is an annual aromatic herb belonging to the *Lamiaceae* family [9]. The centres of origin of sweet basil are the tropical and sub-tropical regions of Africa, Asia and South America [10]. Sweet basil, which is a is a frost tender plant, can be produced easily in fertile, well drained but moist soil with a pH of 5.5–7.0. Optimum temperature for germination is 70 °F (20 °C), but the

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seeds will germinate well between 6585 °F (1530 °C) in about 7 days and it can be grown year-round in tropical and subtropical regions in well-drained soils at elevations below 700 ft (250 m) [11]. The fresh-tasting, fragrant leaves of the sweet basil plant are a desirable ingredient in the cuisine of several regions, including Asian and Italian. In traditional medicine, *O. basilicum* L. has been used to treat headaches, coughs, diarrhoea, constipation, warts, worms, and kidney malfunction [10]. The extracted oil from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics [12]. The seeds of sweet basil, however, are mainly used for propagation of the crop.

Biodiesel (which consists primarily of fatty acid esters) is typically produced by transesterification of triglycerides using short-chain alcohols, such as methanol and ethanol. Glycerol is the main by-product of biodiesel production. ASTM (American Society for Testing and Materials) D6751 and European standard EN 14214 are the general standards for commercial biodiesel and the physicochemical properties of biodiesel generated from any feedstock must meet these minimum standards for commercial sale and application.

Several parameters can affect the biodiesel yield and physicochemical properties; therefore, optimizing the production parameters using mathematical methods is necessary to minimize the process cost and maximize production efficiency [13]. The artificial neural network (ANN) approach to process optimization is a well-known evolutionary computational method used widely in the last decades. ANNs are nonlinear computer algorithms that perform a black box modelling based on the biological learning process of the human brain and contains a number of parallel layers of neurons, all connected by weighted links [14]. Various studies have demonstrated that the combination of ANN and genetic algorithm (GA) is powerful technique to solve the optimization problems [15,16].

The aim of this study was to investigate a novel feedstock for biodiesel production using lipase-catalyzed transesterification and to optimize the production. Oil was extracted from basil seeds (BSO) and its physicochemical properties assessed to determine suitability as a biodiesel feedstock. BSO was then converted to biodiesel using lipase-catalyzed transesterification and the properties of the resulting biofuel were compared to EN 14214 and ASTM 6751 standards specifications. Finally, the lipase-catalyzed transesterification process was optimized using ANN and GA modelling. To the best of our knowledge, the present study is the first to investigate biodiesel production from BSO and provides insights into the utility of this alternative biofuel feedstock.

2. Materials and methods

2.1. Reagents and chemicals

O. basilicum seeds were collected from an herbalist in Iran. n-hexane (99% AR grade), methanol, Novozym 435 (containing recombinant lipase from *Candida Antarctica* immobilized in acrylic resin, Sigma Aldrich, USA), extraction thimbles (30 mm × 100 mm), methyl nonadecanoate (Sigma Aldrich, USA), and FAME mixture C_8–C_{28} (Supelco) were purchased from Chemolab Supplies (Kuala Lumpur, Malaysia).

2.2. Oil extraction

Basil seeds were cleaned and sun dried for one week. The seeds were finely ground with a waring blender and the oil was extracted using n-hexane at an 8:1 solvent to sample ratio for 4 h. Hexane and water were then evaporated by rotary evaporation at 60 °C and the oil was filtered to remove solid impurities. The yield of the oil was calculated according to the following equation;

$$\text{Oil Yield }(\%) = \frac{\text{mass of extracted oil (g)}}{\text{mass of basil seeds (g)}} \times 100\% \qquad (1)$$

Basic physicochemical properties of the basil seeds oil (BSO), including viscosity, density, acid value, calorific value, oxidative stability, pour point, and cloud point were determined as per EN 14214 or ASTM 6751 testing methods.

2.3. Biodiesel production

Biodiesel was produced from BSO using immobilized lipase (Novozym 435) and methanol. The reaction was carried out in 100 ml Schott bottles using the *Labtech* shaking incubator model LSI-3016A and the optimal conditions for biodiesel production were as follows: 47 °C, 6% w/w immobilized lipase, 68 h reaction time, and methanol to oil ratio 11:1. Methanol was added at three time intervals to maintain methanol to oil ratio.

2.3.1. Product purification and separation

Immobilized lipase was separated from the reaction mixture by filtration and subsequently washed sequentially with distilled H_2O and tert-butyl alcohol, freeze-dried, and retained for reuse. The reaction mixture was centrifuged at 8000*g* for 10 min to separate FAMEs from the glycerol. Glycerol was removed and FAMEs were mixed with distilled water at 40 °C to remove residual impurities from the biodiesel. Methanol and water were removed by rotary evaporation at 65 °C.

2.4. Analytical methods

Crude BSO and purified FAMEs were analyzed by Fourier transform infrared spectroscopy (FTIR) as per ASTM D7418 from 400 to 4000 cm^{-1}. FAMEs were identified by gas chromatography (Agilent 7890, California, USA) equipped with FID (flame ionization detector). The physical properties of the crude oil and FAME were determined according to standard testing methods of American standard (ASTM D6751) and European Union standard (EN 14214). Effect of parameters on FAME conversion, including methanol to oil molar ratio, catalyst concentration, reaction temperature and reaction time were studied. Fatty acid methyl esters and the total fatty acid methyl esters obtained after 24 h, 48 h and 72 h were calculated according to the following equation;

$$\text{FAME conversion }(\%) = \frac{\sum A}{A_{EI}} \times \frac{Wt_{EI}}{m} \times 100\% \qquad (2)$$

where $\sum A$ is the area of all peaks in chromatogram (C_6–$C_{24:1}$), A_{EI} is the area under the curve for the internal standard (C_{19}), Wt_{EI} is the weight of the C_{19} internal standard (mg) and m is the sample weight (mg).

2.5. Artificial neural network (ANN) – genetic algorithm (GA) modelling and optimization

MATLAB's Neural Network and Genetic Algorithm Toolboxes (MATLAB 8.1.0.604) were applied in modelling and the optimization of the FAME content in biodiesel. A feed forward, back-propagation multilayer ANN was performed using the Levenberg–Marquardt (LM) algorithm with a hyperbolic tangent sigmoid (tansig) transfer function from the input to the hidden layer and a purelin transfer function from the hidden layer to the output. The selected ANN had an input layer with four neurons (temperature, catalyst loading, reaction time, and methanol to oil ratio), a hidden layer, and an output layer with one neuron (FAME content). The