Industrial Crops and Products 52 (2014) 430–438

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Artificial neural network modeling of biolubricant production using Novozym 435 and castor oil substrate

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ARTICLE INFO

Article history:
Received 8 September 2013
Received in revised form
10 November 2013
Accepted 11 November 2013

Keywords:
Novozym 435
Biolubricant
Castor oil
Enzymatic methanolysis
ANN modeling
NMR analysis

ABSTRACT

Industrial attention on the seeds of *Ricinus communis* plant is mainly due to its hydroxylated fatty acid content. With use of Novozym 435 the methanolysis of castor oil for biolubricant synthesis was studied. Modeling of this production was performed using artificial neural network (ANN), where the architecture of the neural net was built by incorporating the four test factors as the input layer, methyl ester yield as the response variable, and one hidden layer with different numbers of the constructed neurons. The final selected model (4-11-1) with considering appropriate transfer function and learning rule, exhibited a good prediction capability with a high linear correlation coefficient of 0.9964 and a low mean squared error for the data testing. NMR analysis on the extracted castor oil and its methyl ester derivative were used to characterize the progress of the reaction. Methyl ester yield around 98% was obtained for methanol to the oil molar ratio of 6 using 5% of the enzyme at 42 °C after 39 h of the reaction. The Novozym 435 was reusable and performed effectively in castor oil conversion as shown in the repeated batch experiments. The produced biolubricant was evaluated based on the standardized physicochemical properties.

1. Introduction

Petroleum-derived lubricants are mineral based products having limited use because of their toxic nature and lack of biodegradability. This has intensified researches efforts on finding a suitable substitute and because of the process economics and renewability character of biological sources, special attentions have directed toward biotechnological methods (Madankar et al., 2013a; Salih et al., 2011; Gryglewicz et al., 2013). In cases of biodiesel and biolubricant productions attentions are directed toward non-edible oily substrates (Hama and Kondo, 2013).

Oily fraction of *Ricinus communis* seeds contains large amount of the ricinoleic acid as a hydroxylated fatty acid and this structural property has given a unique place to this biological source for industrial synthesis of the variety of compounds (Mutlu and Meier, 2010). For instance use of biolubricants has several advantages, maintenance of engines lubricity is essential for its proper performance and the reduction in the lubricity due to the use of low-sulfur containing petrodiesel causes real damages to the engines. High viscosity character of castor oil increases chance of castor oil methyl ester (COME) usage in blending with petrodiesel for several industrial applications such as lubricity enhancer and also viscosifiers in preparing oil drilling fluids (synthetic-based muds) (Madankar et al., 2013b; Caenn and Chillingar, 1996). With considering role of lipases then enzymatic production of biolubricant takes special reaction characteristic.

Lipases as the enzymes of industrial interests are involved in a variety of the catalytic reactions such as hydrolysis, esterification, interesterification, and alcoholysis. Enzymatic processes are consumed less energy and proceed under mild conditions; there are also other advantageous points for these biotechnological processes (Gandhi, 1997; Narwal and Gupta, 2013). In biolubricant production, the focusing point is on the catalytic role of lipase in the ester synthesis using methanol as an alcoholic substrate and with considering the other substrate which is oily in nature, the esterification reaction proceeds in a less polar environment (Antczak et al., 2009; Kojima et al., 2004). Hydrophilicity character of the solvent increases the likeliness of disruption of the hydrogen bonding and hydrophobic interaction around the enzyme molecule in the reaction solution, these structural disorderness eventually lead to the lipase conformational change (Soumanou and Bornscheuer, 2003; Liu et al., 2011).

Artificial neural networks (ANNs) are robust computational tools in handling the problems especially in biotechnology subject with less clear underlying theory but with good data accessibility. One or more of the following ANN features may involve in ANN classification: the direction of flow of information within the network, connectivity of the neurons in the network and its extent, type of learning algorithm, and learning rule (Basheer and Hajmeer, 2000; Agatonovic-Kustrin and Beresford, 2000). Bioprocesses modeling

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http://dx.doi.org/10.1016/j.indcrop.2013.11.020

and systems' predictability could substantially be improved with use of ANNs.

The objective of the present work was to evaluate predictive capability of ANN for synthesis of biolubricant using Novozym 435 with the castor oil and methanol substrates. Transesterification process was performed with considering reaction time, enzyme amount, substrate molar ratio, and temperature as the input variables and the methyl ester yield as the output variable. Works were directed to characterize the COME using ^{1}H and ^{13}C NMR spectroscopy analyses and further tests were carried out to evaluate the physicochemical properties of the COME as a biolubricant.

2. Materials and methods

2.1. Materials

Novozym 435 (immobilized form of lipase B from *Candida antarctica*) was provided as a gift from Novozymes A/S (Denmark – Tehran office). The seeds of *R. communis* used for castor oil extractions were obtained from the local supplier. All chemicals used in the present study were of analytical grade and purchased from local market.

2.2. Castor oil extraction and analysis

The castor seeds after dehulling were ground into fine particles at 1–2 mm in size. The oil was then extracted using laboratory Soxhlet apparatus with n-hexane as the solvent. After extraction for 6 h, the solvent was distilled off in a vacuum rotary evaporator at 60 °C and the percentage of oil extracted was determined. The fatty acid composition of the oil extracts was determined by gas chromatography (Acme 6000M, Younglin, South Korea) with flame ionization detector and capillary column (60 m × 0.25 mm × 0.2 μm, TR 882162, Teknokroma, Spain). The oven temperature was kept at 175 °C for 10 min, raised to 200 °C at 10 °C/min and maintained at this temperature for 20 min. Hydrogen was used as a carrier gas at a flow rate of 1 ml/min. The temperatures of the injector and detector were set at 250 °C and 260 °C, respectively. Physicochemical properties of the extracted castor oil were determined according to the official methods of the American Oil Chemists' Society (AOCS, 1998).

2.3. Enzymatic methanolysis reaction

The transesterification reaction of castor oil with methanol was performed in a 30 ml screw-capped bottle with shaking at 200 rpm in some selected temperature (orbital shaker incubator, Heidolph, Unimax 1010) for a specified time of reaction. Defined amount of castor oil (5 g) with different levels of methanol in 15 ml n-hexane (different molar ratios of methanol to castor oil) were the contents of the reaction mixture. The methanolysis reaction was initiated by adding various amount of Novozym 435. At the end of reaction, the enzyme was separated out from reaction mixture and the solvent was removed under reduced pressure of 300 mbar at 60 °C (BUCHI, Rotavapor R-205, vacuum controller V-800). The esterified mixture was centrifuged at 8000 rpm for 10 min (Heraeus-Biofuege Stratos) and the methyl ester present in the upper layer was separated from the lower fraction containing glycerol.

2.4. Yield measurement and product characterization

Determination of methyl ester yield was through use of ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy and the relevant spectra were recorded on a Bruker 500 MHz instrument (UltraShield Plus) using $CDCl_3$ (deuterated chloroform) as the solvent and TMS (tetramethylsilane) as the internal standard. ^{13}C NMR spectra were recorded on the same spectrometer operating at 125 MHz. Details for NMR analyses are given elsewhere with considering the following equation for the calculation of the yield (Hajar et al., 2009; Gelbard et al., 1995):

$$Y = 100 \times \left(\frac{2A_{ME}}{3A_{CH_2}} \right)$$

where Y is the methyl ester yield, A_{ME} and A_{CH_2} are the areas of the methoxy and the methylene protons, respectively. The synthesized methyl ester of castor oil was characterized for its physicochemical properties using American Society for Testing and Materials (ASTM) methods.

2.5. ANN-based design of experiment: data analysis, and model development

The multi-layer perceptron (MLP) as the most commonly used neural net for the learning process consisted of highly interconnected three layers: an input layer, one or more of hidden layers containing a number of the constructed neurons, and output layer. The values assigned to the initial inputs connectors as the weight, are propagated forward through the network and calculations are made based on comparison against some known output value. Error term as the differences between these values for every observation is propagated back through the network and by forwarding the data and back-propagating them iteratively, the system's errors are reduced (i.e., error distribution through the network). These repeated cycles to the neural net are termed the training phase and by passing from this phase, the data are partitioned into two distinct subsets (cross-validation and testing). In fact when the training is complete, the ANN has gained capability of predicting the output upon receiving any input similar to the pattern that it has learned. The effectiveness of the pattern developed can be determined by the testing phase in which comparisons are made without considering an output data (Basheer and Hajmeer, 2000; Glassey et al., 1994). The Levenberg–Marquardt algorithm, with the combined form of linear descent and Gauss–Newton methods, could participate in minimization of the mean squared error (MSE) and the obtained curve thus best predicts response from input variables (Adnani et al., 2011). The feed-forward ANN architecture shown in Fig. 1 has been used in the present study, where the information received from inputs and the other nodes were treated through use of transfer function (the neuron dynamics). Transfer function describes the ability of ANN in transferring the weighted sum of all signals from input, output, including a bias to relevant neuron(s). Sigmoid as the most widely used non-linear function possesses a continuity and differentiability characters on the requirements in the learning process, thus training phase of the ANN is rigorously applied. The summary of network architecture used in the present study and the details of the three main phases in ANN processes (training, cross-validation, and testing) are shown in Tables 1 and 2, respectively. The variables used

Table 1
Summary of the network architecture used in the present study.

Characteristics	
Network type	Multi-layer perceptrons (MLPs)
Number of input	4
Number of output	1
Number of hidden layers	1
Number of hidden neurons	1–15
Transfer function	Sigmoid-Axon
Learning rule	Levenberg–Marquardt
Maximum epochs	1000
Number of training runs	3