ARTIFICIAL NEURAL NETWORK ANALYSIS OF IMMOBILIZED LIPASE CATALYZED SYNTHESIS OF BIODIESEL FROM RAPESEED SOAPSTOCK

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Abstract: Refined vegetable oils are the predominant feedstocks for the production of biodiesel. However, their relatively high costs render the resulting fuels unable to compete with petroleum-derived fuel. Artificial neural network (ANN) analysis of immobilized Candida rugosa lipase (CRL) on chitosan catalyzed preparation of biodiesel from rapeseed soapstock with methanol was carried out. Methanol substrate molar ratio, enzyme amount, water content and reaction temperature were four important parameters employed. Back-Propagation algorithm with momentous factor was adopted to train the neural network. The momentous factor and learning rate were selected as 0.95 and 0.8. ANN analysis showed good correspondence between experimental and predicted values. The coefficient of determination (R2) between experimental and predicted values was 99.20%. Biodiesel conversion of 75.4% was obtained when optimum conditions of immobilized lipase catalysed for biodiesel production were methanol substrate molar ratio of 4.4:1, enzyme amount of 11.6%, water content of 4% and reaction temperature of 45℃. Methyl ester content was above 95% after short path distillation process. Biodiesel conversion was increased markedly by neural network analysis.

Keywords: rapeseed soapstock, artificial neural network, biodiesel, immobilized lipase

1. INTRODUCTION

Biodiesel, namely fatty acid alkyl esters, has become a new kind of clean burning fuel that can be used as a mineral diesel substitute for engines produced from renewable sources such as vegetable oils and fats which are mainly constituted by triglycerides(Marchetti et al., 2007; Michael et al., 2005). The major problem for production of biodiesel is the price of raw material, which accounts for about 70% of the total costs. Therefore, researchers are always looking for the suitable materials to produce biodiesel on a larger scale. Soapstock(SS), a byproduct of the refining of vegetable oils, is an important biodiesel feedstock. SS is generated at a rate of about 5% of the volume of crude oil refined, which consists of a heavy alkaline aqueous emulsion of lipid(Jon et al,. 2005; Ma et al,. 1999; Haas et al,. 2000) There are several uses for this waste, such as the production of soaps or acid oil, and more recently the production of biodiesel. Many attempts have been made to develop an enzymatic process using lipase as catalyst(Haas et al,. 2003; Shashikant et al,. 2005; Shao et al,. 2006).

However the components of SS were so complicated that it was difficult to obtain better results when lipase used in production of biodiesel. The optimization processes involved labor-intensive and low conversion of fatty acid methyl ester. Artificial neural network (ANN) was a mathematical algorithm which had the capability of relating the input and output parameters without requiring a prior knowledge of the relationships of the process parameters(Balaraman et al,.2005). This meaned a short computing time and a high potential of robustness and adaptive performance. The ANN was able to model chemical processes based on linear or non-linear dynamics.

ANN was now the most popular artificial learning tool in biotechnology, with applications ranging from pattern recognition in chromatographic spectra and expression profiles, to functional analyses of genomic and proteomic sequences. The use of advanced non linear data analysis tools such as ANN was often used in food science (Shao et al,. 2007; Montague et al,. 1994). Lou et al. used an artificial neural network (ANN) method, computer model system, which match the functionality of the brain in a fundamental manner, represented the nonlinearities in a much better way (Lou et al,. 2001). Few studies on the optimization of predicting biodiesel conversion from SS have appeared in the literature. There appeared to be a need for the optimization of lipase synthesis for production of biodiesel by ANN analysis.

In this study, it involved first saponification of the rapeseed soapstock followed by acidification to produce free fatty acids. An artificial neural network analysis of immobilized lipase on chitosan catalyzed preparation of biodiesel from rapeseed soapstock was carried out. This would be very interesting for further applications of biochemical processes on a larger scale,

which do not require any kind of mechanistic premises but only input and output variables.

2. MATERIALS AND METHOD

2.1 Materials and reagents

Candida rugosa lipase was purchased from Sigma Chemical Co. (USA). The activity was measured by titrating FA liberated from olive oil with 50mM KOH as described previously. One unit was defined as the amount of enzyme that liberated 1μmol FFA per minute.

Chitosan powder was obtained from Golden Shell Biochemical Co.Ltd. The material was obtained from prawn's shells with a degree of deacetylation of 85%. Glutaraldehyde was purchased from Fluka.

All other chemicals and reagents used were of analytical grade.

Rapeseed soapstock was supplied by Hangzhou Oil and Fat Co. Ltd. (Hangzhou, China).

Pope2# wiped-film molecular still (Pope Scientific, Inc. USA).

2.2 Immobilized of lipase to chitosan beads

Chitosan powder of 3% (w/v) was completely dissolved in 1% (v/v) acetic acid. This solution was poured into a coagulant bath of 1N sodium hydroxide solution containing 26% (v/v) ethanol under stirring to form spherical gels and allowed to stand for 3h. chitosan was obtained by filtration and rinsed with distilled water until neutrality(Foresti et al,. 2007). One gram of chitosan beads were mixed with 3mL of 0.01% (v/v) glutaraldehyde was added to the beads. After 20min, the supernatant was removed and 3ml of 0.5% (v/v) lipase in deionized water was added to the beads and allowed to react for 45min. Finally, the beads were washed thrice in deionized water. The beads were resuspended in deionized water and stored at 4℃.

2.3 Saponification and acidification

Saponification of soapstock was carried out with the use of 0.5 M sodium hydroxide followed by acidification. Acidification of SS was essential for the reaction, which destroy the emulsion of the complicated mixtures. SS was acidified to acid oil in a 500ml round bottom flask, which required the addition of sulfuric acid. The pH of the mixture was adjusted to 2-3 so as to

fully convert the soap to fatty acid. In addition, the acidification was carried out at high temperature of 90℃ for 1.5h. The mixture was settled in separation funnel to remove the bottom fraction, then top was washed to neutrality and dried. Its FFA content was determined by a standard titrimetry method. This acid oil had an initial acid value of 186 mg KOH/g corresponding to a FFA level of 96%.

2.4 Immobilized lipase catalyzed reaction

Experiments were conducted in a laboratory-scale setup which consisted of 100ml glass flasks with condensation tube. The flasks were kept in a water bath maintained at some temperature. Immobilized lipase was added the mixture of oil and methanol. The molar amount of the oil was calculated from its saponification value. The contents were stirred after tightly closing the tube. The reaction conditions were optimized by ANN analysis. At end of reaction, the enzyme was separated out by filtration and filtrate was washed with distilled water after transferring it to a separating funnel. The ester phase was then dried using anhydrous sodium sulfate and the solvent was removed under reduced pressure.

2.5 Distillation of crude fatty acid methyl esters

The product of the above mentioned reaction was washed and dried for the sake of the distillation using short path distillation(Jiang et al,. 2006). The conditions were determined as follows: evaporating temperature 110℃ at 5.32 Pa, rolling speed of 150 min-1 and feed temperature of 80℃. The light component was the fatty acid methyl esters we required. GC-MS was implemented to characterize the biodiesel.

2.6 Determination of FAME and saponification value

Saponification value was determined by standard method.

FAME composition was determined by the procedure(Shao, et al,. 2007).

2.7 Artificial neural network model

There were various types of ANNs. The type chosen for use in this study was the back propagation learning algorithm that was very powerful in function optimization modeling. In these networks, signals were propagated from the input layer through the hidden layers to the output layer. A node thus received signals via connections from other nodes, or the outside world in the case of the input layer. To train an ANN model, a set of data

containing input nodes and output nodes are fed. Once the training was over, ANN was capable of predicting the output when any input similar to the pattern that was has learned was fed. The ANN was tested for the remaining set of experimental data (Balaraman et al,. 2005).

Artificial neural network (ANN) was applied here comprises of three layers to provide a nonlinear mapping, in the BP network used here, the input consists of methanol substrate molar ratio, enzyme amount, water content and reaction temperature. The central composite design was showed in Table 1. All experiments were carried out in a randomised order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Table 1 Independent variables and their levels for central composite design.

Independent variables	codes	variable levels		
		-1	0	+1
methanol molar ratio	X_1	2:1	4:1	6:1
enzyme amount(%)	X_2	4	8	12
water content(%)	X_3	2	6	10
reaction temperature(℃)	X_4	35	45	55

The node number of network's input layer (iN) was set at 4 and the node number of output layer was oN = 1 corresponding to biodiesel conversion. Each layer had a bias except output layer.

According to the dimension of samples, the training precision should be improved to avoid the training progress becoming "under-fitting". Large values of training epochs, hidden layer's node number (hN) and learning rate (lr) were imported to reach this aim. They were set as epochs = 10000, hN = 6 and lr = 0.8. So the number of network's weights that need be identified was decided(Haykins 1994):

$$W = (iN + 1)\, hN + (hN + 1)\, oN = (4 + 1)\, 8 + (8 + 1)\, 1 = 49 \quad (1)$$

The number of sample subset for training was assumed as Ntrain and the number of sample subset for test was assumed as Ntest .We could calculate the ratio between Ntrain and Ntest (ropt) just using the equation:

$$r_{opt} = \frac{N_{train}}{N_{test}} = 1 - \frac{\sqrt{2W - 1} - 1}{2W - 2} \quad (2)$$

Now the total number of samples is known :

$$Ntrain + Ntest = 27 \quad (3)$$

Computing Equation (1), (2) and (3), it was easily gotten: Ntrain = 24.23. Because the number should always be an plus integer, the value was changed as Ntrain = 25, which means we should choose 25 units of samples as the training samples and the other 2 units as testing samples.

At the same time, to avoid the training progress becoming "over-fitting", a method called as estimation-followed-by-validation was introduced. The principle of this method was that after each epoch of network's training, samples for testing were imported to validate which the training progress was over-fitting or not.

Back-Propagation algorithm with momentous factor was adopted to train the neural network. Here, the momentous factor was selected as mom = 0.95. Random value in the range [-1, 1] was used as the initial weight of network. Mean Square Error (MSE) was chosen as the performance function. In ANN computation, we used the toolbox Matlab 6.5.

ANN tends to implicitly match the input vector to the output vector. ANN had been applied for the purpose of simulation on the same experimental data used for ANN analysis (Table 2).

Table 2 Central composite design and experiment data.

Run	X_1	X_2	X_3	X_4	True model (%)	ANN model (%)	Error (%)
1	-1	-1	0	0	40.30	42.251	4.6176
2	-1	1	0	0	42.60	42.298	0.7099
3	1	-1	0	0	38.10	38.038	0.1619
4	1	1	0	0	55.80	53.225	4.6148
5	0	0	-1	-1	43.20	44.653	3.3630
6	0	0	-1	1	47.40	45.711	3.5625
7	0	0	1	-1	54.80	55.039	0.4362
8	0	0	1	1	53.70	53.174	0.9789
9	-1	0	0	-1	45.60	45.217	0.8391
10	-1	0	0	1	47.90	48.131	0.4830
11	1	0	0	-1	50.40	49.766	1.2584
12	1	0	0	1	53.50	55.636	3.9930
13	0	-1	-1	0	35.90	35.288	1.7054
14	0	-1	1	0	42.70	41.892	1.8934
15	0	1	-1	0	34.20	33.151	3.0671
16	0	1	1	0	49.70	49.857	0.3168
17	-1	0	-1	0	38.20	39.307	2.8971
18	-1	0	1	0	46.10	46.143	0.0931
19	1	0	-1	0	36.40	37.279	2.4146
20	1	0	1	0	48.70	48.591	0.2231
21	0	-1	0	-1	42.20	42.843	1.5243
22	0	-1	0	1	42.40	43.146	1.7589
23	0	1	0	-1	44.10	44.295	0.4423
24	0	1	0	1	46.30	47.513	2.6189
25	0	0	0	0	62.90	63.504	0.9602
26	0	0	0	0	64.40	63.504	1.3914
27	0	0	0	0	63.60	63.504	0.1511

3. RESULTS AND DISCUSSION

3.1 Establishment of model

Artificial neural network analysis was employed to solve a wide variety of problems in science and engineering(Montague et al,. 1994). Unlike other modeling techniques such as simultaneous heat and mass transfer, kinetic models and regression models, an ANN could accommodate more than two variables to predict two or more output parameters. ANN differed from conventional programs in their ability to learn about the system to be modeled without a need of any prior knowledge on the relationships of the process variables.

After neural networks were trained successfully, all domain knowledge extracted out from the existing samples was stored as digital forms in weights associated with each connection between neurons. The relationship of the model was expressed as $Y = purelin\ (W21Tansig\ (W11\ p_1 + b1) + b2)$. Fig.1 illustrated the scheme of three layers neural network. Comparison of experimental data with simulated data was shown in Fig.3. The coefficient of determination (R2) between experimental and predicted values was 99.20%. The connection weights value (W11) and bias value (b1) between input layer and hidden layer, the connection weights value (W12) and bias value (b2) between hidden layer and output layer were as follows, respectively.

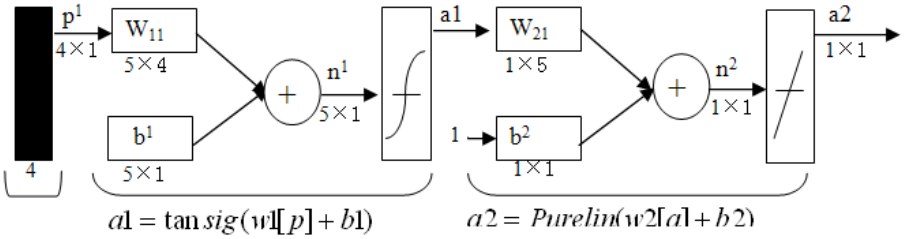

Fig.1. The scheme of three layers neural network.

W11=[0.6791 -0.1797 -1.1696 -0.0430; 0.5828 -1.8350 1.4063 0.0115; 1.5787 0.9839 -0.5267 -0.9566; -1.3268 1.2835 -0.4042 0.9861; 1.7453 -2.6483 0.6485 -0.0039]T

W21=[-0.2203 -0.1000 0.0438 0.0543 0.1562]

b1=[-1.2544 -0.7808 0.3163 -0.1443 1.2315]T

b2=[0.2372]

The effects of methanol concentration on extent of predicted conversion was shown in Figure.2 (A). It was indicated that the methyl ester yield was sensitive to the methanol molar ratio. An increase in methyl ester yield was observed with the increasing of methanol molar ratio at first. But the trend was reversed when the ratio reached a certain value. It could be interpreted that, under a certain amount, the methanol was used to improve the solubility of water in oil and reaction mixture would become well mixed. So with the increasing of methanol amount, methyl ester first increased and then decreased as a result of the decrease of enzyme activity caused by excessive methanol. The effect of enzyme amount on extent of predicted conversion was shown in Figure.2 (B). The biodiesel conversion increased with the increased enzyme amount because of improvement of lipase activity. The optimum methanol molar ratio and enzyme amount for the maximum methyl ester conversion was around 4.4:1 and 11.6%, respectively.

Influence of water content on production of methyl ester showed significant variation both above and below the optimum values (Fig.2 (C)). With the increase of water, we observed an increase at first and decrease subsequently in the amount of methyl ester; It was likely that Candida rugosa lipase was inactivated by methanol due to insufficient water in the reaction mixture and methyl esterification reaction was inhibited when much water content above 4% (Manohar et al,. 2003). Water content about 4% was therefore suggested. When lipase powder was used as the catalyst, the reaction rate was much lower. In this experiment, the amount of lipase powder used was used with some amount of water.

3.2 Validation of the model

Having gotten the model, optimum analysis can be done on this basis. Through the adjustment of parameters X1, X2, X3 and X4, that the value of biodiesel conversion get the maximum is our interest. In the application, all these parameters are put into the network's input layer, but three parameters are fixed and only the other parameter is adaptable. Before optimum, we choose an experiment data as initial data. The simulation shows that when X1 = 0.2, X2 = 0.9, X3 = -0.5 and X4 = 0, biodiesel conversion has the maximum value 0.7720.

According to ANN result, an experiment with an methanol substrate molar ratio of 4.4:1, enzyme amount of 11.6%, water content of 4% and reaction temperature of 45℃ was conducted in order to investigate the effect of ANN. The experiment was carried out at the optimized conditions. Methyl ester conversion of 75.4% was obtained and was in good agreement with the predicted one. The accuracy of the model was validated with triplicate experiments under the aforementioned optimal reaction conditions. As a

result, the model was considered to be accurate and reliable for predicting the conversion of methyl ester.

According to previous result, an experiment with molecular distillation was conducted in order to obtain high content of methyl ester. The content and recovery of biodiesel was above 95%, 89.5% after short path distillation respectively.

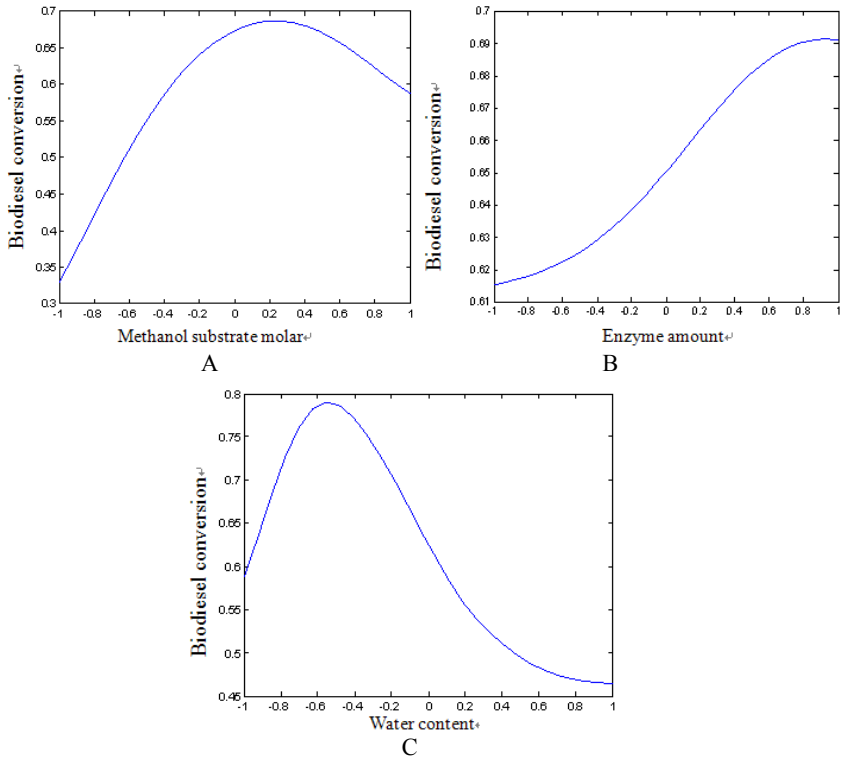

Fig.2. The effects of methanol substrate molar (A), enzyme amount (B) and water content (C) on extent of predicted conversion.

4. CONCLUSIONS AND FUTURE WORKS

From the above analysis, the main outcomes can be outlined as follows:

（1）Artificial neural network was used to optimize the production of biodiesel. The coefficient of determination (R2) for the model is 99.20%. Biodiesel conversion of 75.4% was obtained when optimum conditions of immobilized lipase catalyzed for biodiesel production were methanol substrate molar ratio of 4.4:1, enzyme amount of 11.6%, water content of 4% and reaction temperature of 45℃.

（2）Methyl ester content was above 95% after short path distillation. Validation experiments verified the availability and the accuracy of the model. The predicted value was in agreement with the experimental value. （3）Future work is using other lipases or combined with Candida rugosa lipase to develop an integrated reaction system for production of biodiesel with low cost.

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