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Performance evaluation of artificial neural network coupled with generic algorithm and response surface methodology in modeling and optimization of biodiesel production process parameters from shea tree (*Vitellaria paradoxa*) nut butter

Odedele Olatunde Stephen
Obafemi Awolowo University, Nigeria

This work investigated the potential of shea butter oil (SBO) as feedstock for synthesis of biodiesel. Due to high free fatty acid (FFA) of SBO used, response surface methodology (RSM) was employed to model and optimize the pretreatment step while its conversion to biodiesel was modeled and optimized using RSM and artificial neural network (ANN). The acid value of the SBO was reduced to 1.19 mg KOH/g with oil/methanol molar ratio of 3.3, H_2SO_4 of 0.15 v/v, time of 60 min and temperature of 45°C. Optimum values predicted for the transesterification reaction by RSM were temperature of 90°C, KOH of 0.6 w/v, oil/methanol molar ratio of 3.5, and time of 30 min with actual shea butter oil biodiesel (SBOB) yield of 99.65% (w/w). ANN combined with generic algorithm gave the optimal condition as temperature of 82°C, KOH of 0.40 w/v, oil/methanol molar ratio of 2.62 and time of 30 min with actual SBOB yield of 99.94% (w/w). Coefficient of determination (R2) and absolute average deviation (AAD) of the models were 0.9923, 0.83% (RSM) and 0.9991, 0.15% (ANN), which demonstrated that ANN model was more efficient than RSM model. Properties of SBOB produced were within biodiesel standard specifications.

odedeleolatunde1989@gmail.com
odedele_olatunde@yahoo.com

Improvement of isobutanol production by a genetically modified *Escherichia coli ΔldhA*

M Ebrahimi[1], Gh Amoabediny[1], A Salehi-Najafabadi[1], M A Amoozegar[1] and **E Salehghamari[2]**
[1]University of Tehran, Iran
[2]University of Kharazmi, Iran

Biofuels synthesized from renewable resources are of increasing interest, because of global energy and environmental problems. Compared to ethanol, isobutanol offers many advantages as a substitute for gasoline due to higher energy content and higher hydrophobicity. *Escherichia coli* is a well-characterized microorganism and its physiological regulation is well studied. However, it does not produce isobutanol as a fermentation product. We are engineering a synthetic pathway in *E. coli* to produce isobutanol. Isobutanol is produced from pyruvate through valine biosynthesis. Therefore, we want to delete *ldhA* (lactate dehydrogenase A) that contribute lactate formation. This deletion will increase the level of pyruvate available for the valin biosynthesis. One strategy for knocking out a special gene is homologous recombination. In this way left and right homologous arms at both side of the gene in *E. coli* genome, were amplified by designed primers including restriction sites at their 5 ends. Left arm primers contain BamHI and EcoRI recognition sites and Right arm primers have HindIII and pstI. The PCR products were cloned separately by TA cloning kit and were confirmed by sequencing. pTZ plasmid containing arms were digested with respective restriction enzymes and the digested arms were cloned in MCS of pUC19 vector one after each other. In next step the kanamycine resistance gene was amplified with the primer which contain XbaI recognition site and it was inserted between left and right arms. When three fragments had been inserted the construct prepared, it was sent to *E. coli* cell and after recombination, kanamycine resistance gene stand instead of gene. Finally this strain could use as a strain which increases isobutanol production in *E. coli*.

ebrahimi93@ut.ac.ir