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Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network

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ABSTRACT

This study investigated the use of Breadfruit Starch Hydrolysate (BFSH) as the sole carbon source for bioethanol production and the optimization of the fermentation parameters. The results showed that the yeast was able to utilize the BFSH with and without nutrient supplements, with highest bioethanol yield of 3.96 and 3.60% volume fraction, respectively after 24 h of fermentation. A statistically significant quadratic regression model ($p < 0.05$) was obtained for bioethanol yield prediction. Response Surface Methodology (RSM) optimal condition values established for the bioethanol yield were BFSH concentration of 134.81 g L^{-1}, time of 21.33 h and pH of 5.01 with predicted bioethanol yield of 3.95% volume fraction. Using Artificial Neural Network (ANN), multilayer normal feedforward incremental back propagation with hyperbolic tangent function gave the best performance as a predictive model for bioethanol yield. ANN optimal condition values were BFSH concentration of 120 g L^{-1}, time of 24 h and pH of 4.5 with predicted bioethanol yield of 4.21% volume fraction. The predicted bioethanol yield was validated experimentally as 4.10% volume fraction and 4.22% volume fraction for RSM and ANN, respectively. Coefficient of Determination (R^2) and Absolute Average Deviation (AAD) were determined as 1 and 0.09% for ANN and 0.9882 and 1.67% for RSM, respectively. Thus, confirming ANN was better than RSM in both data fittings and estimation capabilities.

1. Introduction

Due to increased growth of World population and industrialization, energy consumption has increased tremendously over the last century [1]. The need for alternative fuel, which can solve the problems of finite nature of fossil fuels and their environmental concerns, is therefore vital [2]. A steady decline in crude oil production has led to interest in the utilization of biomass to produce bioethanol [3]. Bioethanol is found to decrease emissions of carbon monoxide and greenhouse gases when used in automobiles as a fuel additive at the rate of 5–10%. It can also replace gasoline additive, methyl tertiary butyl ether (MTBE), which is potentially toxic to human health [4]. Studies on bioethanol have been widely reported from three main types of biomass raw materials, which are starch materials like breadfruit [5,6], cassava [7]; sugar materials like cashew apple juice [8], sorghum juice [9], and finally, lignocellulosic materials such as bagasse, straw and wood biomass [10].

One of the promising ecologically advantageous eco-friendly perennial crops that can be fractionated into food, feed and fuel in Nigeria is the breadfruit (*Artocarpus altilis*), which is grown in Southern Nigeria [11]. It was introduced to Ifewara; Southwestern Nigeria from the Caribbean's and spread to the neighboring villages [12]. Breadfruit is one of the highest-yielding food plants, with a single tree producing up to 200 or more fruits per season. It is a good source of carbohydrate with complementary protein content. The starch content of unripe mature breadfruit pulp is 77% [13]. The breadfruit pulp is made into various dishes; it can be pounded, fried, boiled or mashed to make porridge. It can also be ground into flour and used in bread and biscuit making [14]. In an attempt to add value to the crop, enzymatic hydrolysis optimization of its starch using statistical approach, was carried out in a study by Betiku and Ajala [12]. It has been suggested that breadfruit being an underutilized food crop with short shelf life, could serve as economically viable alternative feedstock for bioethanol production [6]. Although starch from the crop has been hydrolyzed and

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subsequently converted to bioethanol [6], neither the hydrolysis step nor the fermentation process were carried out using Design of Experiments (DOE) approach.

One of the most important stages in a biological process is modeling and optimization to increase the efficiency of the process [15]. The typical method of optimization involves varying one variable at a time and keeping the other constant [16]. This technique is not only laborious but does not also depict the complete effects of the variables in the process and ignores the combined interactions between the variables. In contrast, Response Surface Methodology (RSM) is an empirical modeling technique used to establish the relationship between a set of controllable experimental factors and the observed results [17]. RSM defines the effect of the independent variables, alone or in combination, in the processes. In addition to analyzing the effects of the independent variables, this methodology also generates mathematical model [18]. This tool has been used extensively in many areas of scientific research, such as citric acid production [19], biodiesel production [2], ethanol production [9], lactic acid production [20] and drug research [21]. On the other hand, it is difficult to say that it is applicable to all optimization and modeling studies. It has been reported that the quadratic polynomial equation is not suitable in explaining the effects of pH and substrate concentration on the initial reaction rate of the enzymatic reaction [15]. Similar observations were made in optimization studies on kinetic constants determination for an alkaline protease from *Bacillus mojavensis* and lipase catalyzed incorporation of docosahexaenoic acid (DHA) into borage oil using RSM [22,23].

The past decade has seen a host of data analysis tools based on biological phenomena developed into well-established modeling techniques, such as artificial intelligence and evolutionary computing [24]. Artificial Neural Networks (ANNs) are 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 [25]. ANN is an information-processing paradigm that is inspired by the biological nervous systems, such as the brain, process information. Indeed, ANN is a massively interconnected network structure consisting of many simple processing elements capable of performing parallel computation for data processing. The fundamental processing element of ANN (the artificial neuron, unit or nodes) simulates the basic functions of biological neurons [15]. ANN models have been shown to consistently work better than RSM [15,24].

In this present investigation, RSM and ANN analysis of optimization of bioethanol production were carried out using an underutilized food crop (breadfruit) as a potential substrate. Mathematical models were also developed to predict the bioethanol yield.

2. Materials and methods

2.1. Microorganism and inoculum preparation

Vahine brewing instant dry yeast, a product of Nahozer Ghana Ltd, Accra, Ghana, was used for this work. A known quantity of the brewing instant dry yeast was activated by adding a spatula of the yeast to 150 mL sterile distilled water in a 250-mL Duran flask and was shaken on an environment-controlled incubator shaker (New Brunswick Scientific Co., USA) for 18 h [9]. The yeast was then plated and maintained on yeast peptone dextrose (YPD) agar. The yeast was kept in a refrigerator at temperature of 4 °C with fortnight sub-culturing. For the inocula, the inoculating loop was used to scoop cells from a colony on the agar plate and transferred aseptically into Duran flask (250 mL) containing 50 mL of sterilized medium. The inoculated flask was incubated the incubator shaker at 3.3 Hz and 30 °C for 18–20 h before it was used to inoculate the media for the bioethanol production studies [7].

2.2. Breadfruit Starch Hydrolysate (BFSH) production

Freshly harvested mature but unripe fruits (seedless species) from breadfruit tree were obtained from Ile-Ife, Osun State, Nigeria. The fruits were washed to remove adhering latex and dirt, peeled and afterward milled. Water was added to the slurry followed by filtration using muslin cloth and the filtrate was allowed to settle and then the top water was decanted. The starch obtained was sun-dried to constant weight. The method of Betiku and Ajala [12] was employed to generate the BFSH used in this work.

2.3. Media composition

Fermentation medium used for this study composed of carbon source (BFSH), 5 g L^{-1} of yeast extract, 2 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4.7H_2O$ and 1 g L^{-1} $(NH_4)_2SO_4$ [26]. All media and flasks were sterilized using an autoclave at 121 °C for 15 min.

2.4. Submerged fermentation procedure

For each experiment of bioethanol production, 50 mL of BFSH was measured into 250-mL Duran flask and nutrients were added appropriately. pH of the medium was adjusted with 120 g L^{-1} NaOH and 36.5 g L^{-1} HCl buffer solutions. Subsequently, 5% volume fraction of inoculums size was added aseptically to the flask. The flasks were transferred into the environment-controlled incubator shaker (New Brunswick Scientific Co., USA) at 30 °C and 3.3 Hz. Fermentation was performed for 36 h with 6 h sampling interval.

2.5. Experimental design

A three-level-three-factor Box Behnken Design (BBD) was employed to generate 17 experimental runs by considering BFSH concentration (g L^{-1}), fermentation time (h) and pH (Table 1). ANN has the drawback of requiring large amounts of training data in comparison with RSM [27]. This problem was solved by using statistical experimental design to reduce the number of experiments [24]. The experimental data were divided into two sets: training and testing sets sub-section (Table 2).

2.6. Analytical procedures

Each fermentation sample withdrawn was centrifuged using a Biofuge table centrifuge, Primo R Heraeus (Model 37520, Osterode, Germany) at 152.45 Hz for 5 min and the supernatant obtained was analyzed for BFSH and bioethanol concentrations while the residue was used for biomass concentration determination.

2.6.1. Reducing sugar analysis

The dinitrosalicylic acid (DNS) method [28] was used to determine the concentration of BFSH produced, which was expressed as

Table 1
Coding of experimental factors and levels.

Factor	Symbol	Coded levels		
		−1	0	+1
BFSH (g L^{-1})	X_1	80	120	160
Time (h)	X_2	12	24	36
pH	X_3	4.5	5.0	5.5