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Optimization of enzymatic saccharification of water hyacinth biomass for bio-ethanol: Comparison between artificial neural network and response surface methodology

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ABSTRACT

Response surface methodology (RSM) is commonly used for optimising process parameters affecting enzymatic hydrolysis. However, artificial neural network–genetic algorithm hybrid model can also serve as an effective option, primarily for non-linear polynomial systems. The present study compares these approaches for enzymatic hydrolysis of water hyacinth biomass to maximise total reducing sugar (TRS) for bio-ethanol production. Maximum TRS (0.5672 g/g) was obtained using 9.92 (% w/w) substrate concentrations, 49.56 U/g cellulase concentrations, 280.33 U/g xylanase concentrations and 0.13 (% w/w) surfactant concentrations. The average % error for artificial neural networking (ANN) and RSM were 3.08 and 4.82 and the prediction percentage errors in optimum output are 0.95 and 1.41, respectively, which showed the supremacy of ANN in illustrating the non-linear behaviour of the system. Fermentation of the hydrolysate yielded a maximum ethanol concentration of 10.44 g/l using *Pichia stipitis*, followed by 8.24 and 6.76 g/l for *Candida shehatae* and *Saccharomyces cerevisiae*.

1. Introduction

Recently, a substantial hike in the fossil fuel prices due to the rapid depletion of natural energy sources and human population explosion has kindled immense public cognizance towards global energy security [1]. Factors like global warming, environmental considerations and sustainable growth are encouraging scientists to explore low cost, environment friendly alternative energy sources [2]. In a forage for sustainable replacement of fossil fuels, lignocellulosic biomass derived biofuel can be an alternative renewable energy. Their major advantages are abundant availability, sustainability, recyclability, carbon neutrality and absence of 'food vs. fuel' competition [3]. The residual biomass can also be converted into other value added platform chemicals in a well-integrated biorefinery facility. According to Demirbas [4], integrated biorefinery is an establishment where biomass is converted into fuels, power and value added chemicals with minimum waste generation.

Water hyacinth *(Eichhornia crassipes)* is widely found in tropical countries, like India. It is a noxious weed which rapidly depletes the nutrient and oxygen content of the water, thereby affecting the flora and fauna of the ecosystem. Under favourable conditions, water hyacinth can achieve a growth rate of 17.5 metric tonnes per hectare per day [5]. Large availability of water hyacinth makes it an attractive raw material. Conversion of waste water hyacinth biomass (WHB) to biogas and bio-ethanol has already been explored [6,7].

Conversion of biomass to bio-ethanol mainly comprises of following steps: pre-treatment, saccharification and fermentation. Naïve lignocellulosic biomass is generally recalcitrant to microbial and mechanical degradation, thus rendering it difficult to extract fermentable sugars. Lignin, one of the major components of lignocellulosic biomass, is impediment to enzymatic saccharification [8]. Hence, de-lignification can substantially improve the enzymatic saccharification of the biomass. It has been observed that pre-treatment of WHB with sodium hydroxide is an effective delignification strategy [2].

The major factors that affect the efficiency of the enzymatic saccharification of WHB are substrate concentration, enzyme loading, incubation time and surfactant concentration. The current study has two main objectives, viz. (i) maximizing yield of reducing sugars by enzymatic saccharification to enhance bioethanol production and (ii) comparing the performance of statistical and artificial intelligence-based techniques while optimising process parameters of the enzymatic saccharification of WHB. Traditional 'single-factor-at-a-time' optimisation technique is arduous, time taking and may not assure optimum condition. Hence, Artificial neural network-Genetic algorithm (ANN-GA) and Response surface methodology (RSM) have been implemented to study these interaction effects of the process parameters: substrate

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concentration, enzyme loading and surfactant concentration for maximum yield of reducing sugar during enzymatic saccharification. The optimal condition is verified experimentally and compared to determine the efficiency of both RSM and ANN-GA hybrid technique, which may be the first study on comparison of ANN-GA and RSM for enzymatic hydrolysis of water hyacinth using cellulase and xylanase enzymes to maximise reducing sugar yield for bioethanol production.

2. Materials and methods

2.1. Biomass feedstock

Water hyacinth (*Eichhornia crassipes*) plants were obtained from a pond within the premises of Central Mechanical Engineering Research Institute, Durgapur, India. The shoots and the leaves were initially reduced to a particle size of 2–3 cm and then dried at 106 °C for 6 hours. After drying, the particle size of the biomass was further reduced to 1 mm in a knife mill and stored in air tight containers.

2.2. Alkali pre-treatment of water hyacinth biomass

WHB was delignified by pre-treating the biomass by sodium hydroxide in 250 ml Erlenmeyer flasks with a biomass loading of 10% (w/v), 5% (w/v) concentration of sodium hydroxide, soaking time of 1 hour and treatment time of 10 minutes at 130 °C. The pre-treated sample was neutralised and washed repeatedly and then dried.

2.3. Feedstock compositional analysis

National Renewable Energy Laboratory (NREL) analytical protocol was followed to evaluate the composition of WHB [9]. 72 % (v/v) sulphuric acid was added to 300 mg of biomass and was treated for 1 hour at 30 °C. The acid concentration was diluted to 4% (v/v) with de-ionised water. The diluted mixture was autoclaved at 121 °C for 1 hour. After autoclave the mixture is filtered using 0.2 μm filters for HPLC analysis. The solid residue was used to estimate the acid insoluble lignin.

2.4. Physicochemical characterisation of biomass

Physico-chemical characterisations were performed to examine the changes in the biomass after different stages.

2.4.1. Scanning electron microscopy

Scanning Electron Microscope (SEM) (JEOLJSM-5600) analysis was performed to identify the structural transformation.

2.4.2. X-Ray diffraction analysis

X-Ray Diffraction (XRD) was implemented to determine the crystallinity index (CrI) of the WHB using Shimadzu XRD-6000 diffractometer. The range of the X-Ray Diffractogram is scanned between 10–30° with a step size of 0.0205 using Cu-Kα radiation X-Ray (λ = 1.54 Å) generated at a voltage of 40 kV and 30 mA current. CrI of the sample was calculated as follows [10]:

$$\mathrm{CrI}(\%) = \left[\frac{(I_{002} - I_{14.7^o})}{I_{002}}\right] \times 100 \quad (1)$$

where I_{002} is the maximum intensity at the (002) lattice diffraction at $2\theta = 22.4°$ and $I^{o}_{14.7}$ is the intensity of the background scatter at $2\theta = 14.7°$.

To calculate the crystalline size the following equation was used:

$$D(hkl) = \frac{\lambda\kappa}{\beta_o \cos\theta} \quad (2)$$

where D (hkl) signifies the size of the crystallite (nm), 'κ' is the Scherrer constant (0.94), 'λ' is the X-Ray wavelength (for copper the value of 'λ' is 0.1548 nm), β_o is the full width at half of the maximum height of the reflection at hkl measured at 2θ Bragg's angle.

Degree of crystallinity has been calculated using the following equation [11]:

$$\eta_c = \frac{A_c}{A_a + A_c} \times 100 \quad (3)$$

where η_c is the degree of crystallinity, and A_c and A_a denotes the area of the crystalline and non-crystalline regions respectively.

2.4.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is a powerful analytical technique to examine the functional groups of a polysaccharide. IR spectra were studied using Shimadzu spectrometer (Japan). Samples were prepared by mixing 2 mg of biomass and 198 mg of spectroscopic grade KBr. After grinding, the mixture was pressed to form disks. The spectra were generated with an average scan of 16 scans with a resolution of 4 cm^{-1} within a range of 4000–400 cm^{-1}.

2.4.4. Biomass saccharification

Cellulase from *Trichoderma reesei* and xylanase from *Trametes versicolor* were obtained from Sigma Aldrich®. Enzymatic saccharification of alkali pre-treated WHB were carried out in 50 mM citrate buffer (pH 4.8), at 50 °C in 100 ml stoppered flasks with an agitation speed of 150 rpm for 60 hours. Tween-80 was added as surfactant. The reducing sugars (glucose, xylose, arabinose and mannose) were monitored by 2, 5-dinitrosalicyclic acid method [12]. The hydrolysate was centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant collected was filter sterilised for fermentation experiments.

2.5. Experimental designs and optimisation strategy

2.5.1. Artificial neural network modelling

Artificial neural network (ANN) modelling can be an excellent alternative to Response Surface Methodology (RSM) for solving regression based problems of polynomial non-linear systems. ANN architecture is made of highly interlinked bundles of elements called neurons [13], the connections between the neurons defined by weights (w) and biases (b). The neurons are controlled by a defined transfer and a summing function. The most commonly used transfer functions are: *purelin, log sig and tan sig* [14]. A multi-layer neural architecture consists of input, output and hidden layer. Multi-layer feed-forward neural network also known as multi-layer perceptron (MLP) helps in effective management of the neural architecture while solving non-linear regression models. In this study, the predictive model has been built using substrate concentration (% w/w), xylanase loading (U/g), cellulase loading (U/g) and surfactant concentration (% w/w) as the input parameters, and yield of reducing sugar (mg/g) as the output for the model. The function of the input layer is to present the scaled input data to the hidden layer through weights. The hidden layer then sums up the weighted inputs along with the biases as:

$$\mathrm{sum} = \sum_{i=1}^{n} x_i w_i + \theta \quad (4)$$

where, w_i (i = 1,n) represents the weights of the connection between the neurons of the input and the hidden layer, θ is defined as the bias and x_i signifies the input parameter. An activation function is used to transfer the weighted output to a non-linear domain.

The data set formed after hidden layer operation was considered as the input for the output layer. The final predicted response by the ANN model was generated by the output layer. A mean-squared error function was developed using the predicted response and actual