Optimization of bioethanol production from sorghum grains using artificial neural networks integrated with ant colony

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ABSTRACT

In this study, an artificial neural networks (ANN) model is developed to investigate the relationship between bioethanol production and the operating parameters of enzymatic hydrolysis and fermentation processes. The operating parameters of the hydrolysis process which influence the reducing sugar concentration are the substrate loading, α-amylase concentration, amyloglucosidase concentration and strokes speed. The operating parameters of the fermentation process which influence the ethanol concentration are the yeast concentration, reaction temperature and agitation speed. The desirability function of the model is integrated with ant colony optimization (ACO) in order to determine the optimum operating parameters which will maximize reducing sugar and ethanol concentrations. The optimum substrate loading, α-amylase concentration, amyloglucosidase concentration and strokes speed is determined to be 20% (w/v), 109.5 U/g, 36 U/mL and 50 spm, respectively. The reducing sugar obtained at these optimum conditions is 175.94 g/L, which is close to the average value from experiments (174.29 g/L). The optimum yeast concentration, reaction temperature and agitation speed is found to be 1.3 g/L, 35.6°C and 181 rpm, respectively. The ethanol concentration obtained from the fermentation of sorghum starch by Saccharomyces cerevisiae yeast at these optimum conditions is 82.11 g/L, which is in good agreement with the average value from experiments (81.52 g/L). Based on the results, it can be concluded that the model developed in this study model is an effective method to optimize bioethanol production, and it reduces the cost, time and effort associated with experimental techniques.

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1. Introduction

One of the predominant issues nowadays which need to be addressed is humans’ heavy dependence on fossil fuel energy, which leads to an increase in greenhouse gas emissions (Dharma et al., 2016; Wang et al., 2014). Hence, it is necessary to search for alternative renewable energy sources that are needed to replace fossil are required to solve the existing energy problems. Many studies have been carried out on the production of fuels from biomass such as bioethanol in order to substitute fossil fuels (Guigou et al., 2011). Environmental friendly bioethanol is produce from agricultural biomass (Deesuth et al., 2015; Tabah et al., 2016). Both Brazil and the USA are the major bioethanol producers in the world and these countries are still dependent on edible feedstocks such as sugar cane and corn (Gupta and Verma, 2015). Meanwhile, Thailand and China produce bioethanol from cassava (Manihot esculenta), which is also an edible feedstock (Deesuth et al., 2015).

According to Sebayang et al. (2016) bioethanol can be produced from different kinds of raw materials, which are classified into three categories according to their chemical composition: sucrose-containing feedstocks, starch materials and lignocellulosic materials. An alga is a feedstock considered for the sugars and carbohydrates they contain, which can be fermented to produce ethanol based fuels. However, the use of edible feedstocks for fuel production raises concern on global food security, which hampers the worldwide acceptance of using bioethanol as a fuel. For this reason, much effort is being made to produce bioethanol from non-edible feedstocks such as lignocellulosic and starchy agricultural feedstocks to reduce humans’ dependence on fossil fuels (Aditiya et al., 2016).
Lignocellulosic materials are biomass derived from plants with the main components of lignin, cellulose and hemicellulose. Cellu-
lose and hemicellulose are usually composed of up to two-thirds of lignocellulose and are substrates for second generation ethanol production (Griro et al., 2010). In bioethanol production, ligno-
cellulosic biomass has complex chemical compounds such as cellulose, hemicellulose and lignin which are required to sepa-
rate linkages of different polymers into oligomeric subunits by pre-treatment (Alvira et al., 2010). The purposes of pre-treatment are: to remove structural and compositional impediments into (enzymatic) hydrolysis, increase enzymatic accessibility, to remove lignin in lignocellulosic biomass and to improve the digestibility of cellulose (Anjali and Satyendra, 2014). The bioethanol production from lignocellulosic biomass has been given attention to improve bio-oil quality before further process which related to storage, transport, and final processing characteristics of the oil. Briefly, pre-treatment has been conducted by some researchers. Westerhof et al. (2011) conducted pre-treatment with stepwise pyrolysis of pine wood in a fluidized bed reactor. It was found that stepwise pyrolysis has faster reaction rate compare to the conventional reac-
tion and release compounds from the biomass particle. Moreover, Mourant et al. (2011) investigate the effects of inorganic species in biomass such as K, Na, Mg and Ca on the yield and properties of bio-oil from the pyrolysis of biomass. The result was found that components affected in acid-soluble for bio-oil composition and properties.

1.1. Sweet sorghum feedstock

Sorghum (Sorghum bicolor (L.) Moench) is one of the non-edible feedstocks used to produce bioethanol (Fedenko et al., 2015). It is one of the plants that originated from Africa, but it is now cultivated in Asia (Elhassan et al., 2015; Mehboob et al., 2015). Sorghum (Sorghum bicolor (L.) Moench) is a highly adaptable plant because it can be grown on lowlands and highlands, as well as dry and humid climatic regions (Deesuth et al., 2015). Sorghum can also be grown on marginal lands (mainly arid lands) where other plants are unable to thrive (Elhassan et al., 2015; Wang et al., 2016). For this reason, marginal lands can be utilized for the cultivation of sorghum, which is an advantage since this eliminates competition with lands used for food production (Matsakas and Christakopoulos, 2013).

In addition, sorghum has other advantages such as high grains production and high biomass. Moreover, it is resistant to droughts (Marx et al., 2014; Mehboob et al., 2015; Zhang et al., 2010), it has short regeneration time (3–5 months), it has low fertilization needs and it has higher photosynthetic rate compared to sugar cane and corn (Deesuth et al., 2015; Houxi and Fritschi, 2015; Sjöblom et al., 2015). The starch content of sorghum grains is high (65–71%) and therefore, this starch can be hydrolysed into simple sugars (Dyartani et al., 2015). Moreover, Sweet sorghum juice is better suited for bioethanol production due to higher reducing sugar con-
tent as compared to other sources such as sugarcane juice (Khajil et al., 2015). The important parameters of sweet sorghum such as seed propagation, mechanized crop production, non-invasiveness, biological nitrification inhibition have attracted much attention as a potential non-edible feedstock for bioethanol production. The cost of bioethanol production from sweet sorghum is cheaper as compared to sugarcane molasses at prevailing prices (Ibeto et al., 2011).

As a substitute for gasoline, sorghum bioethanol is widely val-
ued. Few studies have examined on the bioethanol production from sweet sorghum. Guo et al. (2010) investigated optimization and analysis of a bioethanol agro-industrial system from sweet sorghum. Balat and Balat (2009) summarized life cycle analysis of the sweet sorghum ethanol process to evaluate energy efficiency and environmental for different production and use scenarios. There has been a major research effort to investigate storage and continuous supply of sweet sorghum for the bio refinery has been presented in Table 1 (Rao et al., 2009). Therefore, the assessments of environmental sustainability must be addressed to energy and environmental impacts such as land use can be intensive, air, water and soil pollution from the use of fertilizers and plant protection (Gupta and Verma, 2015).

As one of the renewable energy sources derived from non-
edible plants, bioethanol is an alternative fuel which can be used to substitute fossil fuels (Costagliola et al., 2013). There-
fore, studies on bioethanol production have been carried out in pre-treatment, hydrolysis, fermentation and distillation pro-
cess. Currently, conversion technologies for producing bioethanol from biomass feedstock focuses on the development of cellulose-
derived glucose due to enzyme cellululosic and fermentation of sugars derived from starch and sugar. Marx et al. (2014) conducted bioethanol production from sweet sorghum bagasse. Pre-treatment and hydrolysed were performed in a single step using catalyst acid (H₂SO₄) with microwave irradiation assistance. The obtained of ethanol yield was 94% based on the total sugar 480 g/kg during fermentation after 24 h using a mixed S. cerevisiae and Z. Mobilis organisms. On the other hand, Wang et al. (2013) has focused on optimization simultaneous saccharification and fermentation from sweet sorghum using response surface methodology (RSM). They found that the optimum concentration of solid bagasse to be 7% and it can be increased as well as high concentration of solid bagasse. Goshadrou et al. (2011) investigated bioethanol production from sweet sorghum bagasse using zygomycetes fungus Mucor hiemalis. It was treated with H₂SO₄ and KOH which improve the sub-
sequent enzymatic hydrolysis to be 79–92% of the theoretical yield of 81%. Moreover, Khawla et al. (2014) compared acidic hydroly-
ysis and enzymatic hydrolysis at a temperature of 60 °C and pH of 4.5 in which potato peel wastes were used as the feedstock. S. cerevisiae yeast was used during the fermentation process and the pH, reaction temperature and agitation speed was 5.0, 30 °C and 100 rpm, respectively. The fermentation process was carried out over a period of 48 h. The reducing sugar concentration and ethanol concentration was found to be 69 and 21 g/L, respectively. Based on the discussion above, it can be deduced that the produc-
tion of bioethanol by hydrolysis and fermentation gives satisfactory results. However, the production of bioethanol via experiments is both costly and time-consuming since it requires raw materials, time and effort to prepare samples, run the experiments and deter-
mine the optimum operating conditions which will maximize the bioethanol yield. Hence, there is a need to determine the optimum conditions for bioethanol production in a simpler, more efficient manner.

1.2. Artificial neural networks (ANN) modelling

Artificial neural networks (ANN) are an artificial intelligence technique inspired by the human nervous system and it is com-
monly used for modelling and optimizing complex phenomena involving a large number of process variables (Kamairudin et al., 2015; Siswantoro et al., 2016; Vani et al., 2015). Trained ANN can be used as prediction and optimization models for a variety of applications. The prediction capability of the ANN is determined based on experimental data and then validated by independent data (Deh Kiani et al., 2010). ANN can be used to solve non-linear problems by evaluating the relationship between the input and output param-
eters even when the data are complex and incomplete (Mohamed Ismail et al., 2012). ANN has the ability to learn in order to increase production when new data are available (Najafi et al., 2008). There-
fore, ANN is a good alternative to model and optimize the operating parameters of bioethanol production because of its high reliability and efficiency.
Table 1
The availability of bioethanol from sweet sorghum compared to other various biomass feedstocks (Rao et al., 2009).

<table>
<thead>
<tr>
<th>Biomass feedstocks</th>
<th>Cost of cultivation (USD/ha)</th>
<th>Crop duration (months)</th>
<th>Fertilizer requirement (N-P-K/kg/ha)</th>
<th>Water requirement (m³)</th>
<th>Ethanol yield (liters/ha)</th>
<th>Bioethanol production (kg/ha)</th>
<th>Cost of bioethanol production (USD/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet sorghum</td>
<td>217/crop</td>
<td>4</td>
<td>80 – 50 – 40</td>
<td>4,000/crop</td>
<td>4,000/year over two crops</td>
<td>416.67</td>
<td>0.32</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>1079/crop</td>
<td>12 – 16</td>
<td>250 – 400 – 125</td>
<td>36,000/crop</td>
<td>6,500/crop</td>
<td>205.47</td>
<td>–</td>
</tr>
<tr>
<td>Sugarcane molasses</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.372</td>
</tr>
</tbody>
</table>

With the rapid advancement of computer technology, ANN is now widely used in various disciplines of scientific research such as mechanical engineering, chemical engineering and renewable energy (Ayodele and Cheng, 2015; Pelletier et al., 2016; Tasdemir et al., 2011). ANN has been used to determine the optimum operating parameters for hydrolysis and fermentation processes that are critical in bioethanol production.

Ant colony optimization (ACO) is a swarm intelligence technique that is inspired from the foraging behaviour of ant colonies. Integrating ACO with ANN is desirable since ACO is capable of optimizing complex process parameters (Chandra Mohan and Baskaran, 2012; Dorigo and Gambardella, 1997). Due to the laborious and time-consuming methods for determining ethanol yields, recent interest has developed in finding more efficient methods for screening large numbers of samples for bioethanol production potential. Therefore, the aim of this study is to predict and optimize the process of hydrolysis and fermentation in the production of bioethanol from sorghum starch using ANN methodology integrated with Ant Colony Optimisation algorithms. ANN is used to determine the interaction effects of the following operating parameters: substrate loading, α-amylase concentration, amylglucosidase concentration, stroke speed, yeast concentration, reaction temperature and agitation speed. A mathematical model is developed to predict and validate the results of bioethanol production. It is believed that this method will be useful to determine the optimum process conditions for bioethanol production since it will reduce the cost, time and effort required to determine these conditions, which is typically the case with experiments.

2. Materials and methods

2.1. Sorghum feedstock preparation

Sorghum (Sorghum bicolor (L.) Moench) grain was sourced from Cilacap, Central Java in Indonesia. The sorghum grains were first washed and then dried in order to facilitate in peeling the skin before the sorghum grains were used. Following this, the sorghum grains were ground and sieved to obtain starch. The sorghum grains were homogenized, having size smaller than 1 mm (125–150 μm) and it has an effect on reducing sugar concentration in hydrolys- sis process. According to Barcelos et al. (2011) the decrease of the particle size diminished the effect of diffusion limitation and led to a higher sugar yield in comparison with higher particle sizes. The sorghum grains were dried so that they can be stored for longer periods at a temperature of 25°C.

2.2. Microorganism preparation

S. cerevisiae instant dry yeast was purchased from Sigma-Aldrich (Saint Louis, MO, USA). The instant dry yeast was activated by adding 100 mL of sterile distilled water into a 150 mL flask. The yeast was then inoculated in yeast peptone dextrose (YPD) agar in a glass Petri dish. Following this, the yeast was stored in an incubator at 37°C for 24 h prior to use for bioethanol production. α-amylase enzyme from Bacillus licheniformis Type XII-A and amylglucosi-dase enzyme from Aspergillus niger were used as the catalysts for liquefaction and saccharification. Both enzymes were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The enzymatic activity for α-amylase and amylglucosidase was specified to be ≥500 U/g of protein and 300 U/mL, respectively.

2.3. Hydrolysis and fermentation procedure

2.3.1. Hydrolysis

Each sample was prepared in a flask filled with distilled water in accordance with the general procedure for enzymatic hydrolysis. The sorghum starch (10, 15 and 20% (w/v)) was mixed with distilled water and α-amylase (90, 100 and 110 U/g). The samples were placed in a water bath shaker and shaken at different strokes speeds (50, 90 and 130 strokes-per-minute, rpm) at 90°C for 90 min during the liquefaction process. Saccharification was carried out after the liquefaction process using amylglucosidase (36, 51 and 66 U/mL) at 70°C for 240 min. Upon completion of hydrolysis, the samples were cooled to room temperature and the residues were separated from the solution by centrifugation at 10,000 rpm for 5 min. The resultant yellowish liquid (hydrolysate) was isolated for each sample and analysed to determine its sugar content. The raw material was examined to determine the levels of glucose hydrolysed using DNS method. Following this, the enzymatic hydrolysis of sorghum starch was optimized.

2.3.2. Fermentation

The fermentation process was carried out using 250 mL Erlenmeyer flasks filled with the hydrolysed sorghum starch mixed with the following fermentation nutrients: 1 g of yeast extract, 0.4 g of KH₂PO₄ and 0.2 g of NH₄Cl. Each flask was filled with 100 mL of hydrolysed solution and sterilized at a temperature of 125°C and pH of 6.0 for 35 min using an autoclave. The sterilized solutions were then inoculated with S. cerevisiae yeast (0.5, 1.0 and 1.5 g/L). The chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The flasks were then placed into an incubator shaker for the fermentation process. The incubator shaker was set at different temperatures (30, 36 and 42°C) and agitation speeds (50, 150 and 250 rpm) for the fermentation process.

2.3.3. Analysis of reducing sugar and ethanol concentrations

The reducing sugar obtained from enzymatic hydrolysis of sorghum starch was analysed using the 3,5-dinitrosalicylic acid (DNS) method. Glucose was used as the standard (Miller, 1959). The reducing sugar solution was diluted with distilled water up to 1 mL in a test tube. Following this, 1 mL of DNS solution was added into the reducing sugar solution and the mixture was boiled at 90°C for 5 min. UV–vis spectrophotometer (SPEKOL® 1500, Germany) was used to measure the absorbance of each sample at a wavelength of 540 nm. The ethanol concentration of each sample was determined using a gas chromatography system (Agilent 7890A, USA) equipped with a thermal conductivity detector and DB-ALC2 analytical column (30 m × 0.00032 m). Hydrogen (H₂) was used as the carrier gas. The temperature of the injector and detector was 150 and 200°C, respectively. Quantification was carried out by analysing the corre-
sponding peak areas based on the calibration curve prepared from various aqueous ethanol standards.

2.4. Design of experiments

Box-Behnken experimental design was used to generate 29 experimental runs for the hydrolysis process. Four parameters were investigated for the hydrolysis process: (1) substrate loading (% (w/v)), (2) α-amylase concentration (U/g), (3) amylglucosidase concentration (U/mL) and stroke speed (strokes per minute). The Box-Behnken experimental design was also used to generate 17 experimental runs for the fermentation process. Three parameters were investigated for the fermentation process: (1) yeast concentration (g/L), (2) reaction temperature (°C) and agitation speed (rpm). The coded levels of the independent variables used for hydrolysis and fermentation of sorghum starch are presented in Table 2.

2.5. Modelling using ANN

MATLAB 7.10.0 was used to train the backpropagation ANN developed in this study. A three-layer feedforward architecture was used for the ANN model. The hyperbolic tangent sigmoid (tansig) transfer function was used for the input layer to the hidden layer. The purelin transfer function was used for the hidden layer to the output layer. The tansig and purelin transfer function is given by Eqs. (1) and (2), respectively, as follows:

\[
\begin{align*}
tan \, sig(x) &= \frac{2}{(1 + e^{-2x})} - 1 \\
A &= purelin(x) = x
\end{align*}
\]

The backpropagation ANN with Levenberg–Marquardt algorithm was used in this study. The ANN architecture consists of four inputs for the hydrolysis process (i.e. substrate loading, α-amylase enzyme concentration, amylglucosidase enzyme concentration and stroke speed), three inputs for the fermentation process (i.e. yeast concentration, reaction temperature and agitation speed), hidden layers with the optimum number of neurons and a single output variable. The ANN model was trained until the mean square error (MSE) was minimized and the average correlation coefficient was close or equal to 1. The well-trained and tested ANN model was then integrated with ACO in order to predict the reducing sugar and ethanol concentrations at various combinations of operating parameters. The ANN architecture for the hydrolysis and fermentation process is shown in Fig. 1(a) and (b), respectively.

2.6. Ant colony optimization

Ant colony optimization (ACO) is a swarm intelligence technique that is used to solve hard combinatorial optimization problems. This technique is inspired from the foraging behaviour of ant colonies (Chandra Mohan and Baskaran, 2012). In nature, ants will leave pheromone along the route when they leave their nest in order to identify foods by following other members of the colony (Huang et al., 2012). The probability of an ant moving from node k to node l is given by:

\[
Q_{k,l} = \left\{ \left( \sigma_{k,l}^\alpha \right) \left( n_{k,l}^\beta \right) \right\} / \sum \left( \sigma_{k,l}^\alpha \right) \left( n_{k,l}^\beta \right)
\]

where \(\sigma_{k,l}\) is the amount of pheromone on edge \(k,l\), \(\alpha\) is a parameter used to control the influence of \(\sigma_{k,l}\), \(\sigma_{k,l}^\alpha\) and \(n_{k,l}\) represent the desirability of edge \(k,l\) (typically 1/d_{k,l}), and \(\beta\) is a parameter used to control the influence of \(n_{k,l}\).

The amount of pheromone is updated according to the following equation:

\[
\sigma_{k,l} = \Delta \sigma_{k,l} + (1 - \rho) \sigma_{k,l}
\]

where \(\sigma_{k,l}^\alpha\) is the amount of pheromone on edge \(k,l\), \(\rho\) is the rate of pheromone evaporation and \(\Delta \sigma_{k,l}\) is the amount of pheromone deposited.

If ant \(q\) travels on edge \(k,l\), the amount of pheromone deposited is given by:

\[
\Delta \sigma^q_{k,l} = \begin{cases} 
1 & L_q \\
0 & \text{otherwise}
\end{cases}
\]

where \(L_q\) is the cost of the \(q^{th}\) ant’s tour (typically length).

2.7. Verification data

In order to perform a supervised training, one must need to establish a method to evaluate the output error of the ANN model, i.e. the difference between the predicted and actual output. The performance of the ANN model was measured statistically based on the root mean squared error (RMSE) and coefficient of determination (\(R^2\)), which is given by Eqs. (6) and (7), respectively (Prakash Maran et al., 2013; Sarve et al., 2015).

\[
RMSE = \sqrt{\frac{\sum_{i=1}^{n} (y_{oi} - y_{pi})^2}{n}}
\]

\[
R^2 = 1 - \frac{\sum_{i=1}^{n} (y_{oi} - y_{pi})^2}{\sum_{i=1}^{n} (y_{oi} - y_{m})^2}
\]

where \(n\) is the number of experimental data while \(y_{oi}\) is the experimental bioethanol yield, \(y_{pi}\) is the predicted bioethanol yield and \(y_m\) is the average value of the bioethanol yield obtained from the experiments. The accuracy of the model was assessed based on the \(R^2\) and RMSE. In general, the lower the RMSE and the higher the \(R^2\), the more accurate the model. It is recommended that \(R^2\) should not be less than 80%.

2.8. Sensitivity analysis

Calculate the level of significance of input variables for the hydrolysis process (i.e. substrate loading, α-amylase concentration, amylglucosidase concentration and the stroke speed) and input variables for the fermentation process (i.e. yeast concentration, reaction temperature and agitation speed) based on the partition of the relationship between the weights used equation (Garson, 1991). The proposed equation is given by:

\[
pq = \frac{\sum_{r=1}^{r=M_c} \left( \left( \frac{W_{pq}^c}{\sum_{d=1}^{d=M_p} W_{pq}^c} \right) \times |W_{pq}^c| \right)}{\sum_{d=1}^{d=M_p} \left( \left( \frac{W_{pq}^c}{\sum_{d=1}^{d=M_p} W_{pq}^c} \right) \times |W_{pq}^c| \right)}
\]

where \(pq\) is the relative significance of the \(q^{th}\) input variable on the output variable, \(M_p\) and \(M_c\) is the number of input neurons and hidden neurons, respectively, and \(W\) is the connection weight. It shall be noted that the superscript \(p\), \(b\) and \(c\) represents the input, output and hidden layer, respectively, whereas the subscript \(d\), \(s\) and \(r\) represents the input, output and hidden neuron, respectively.
**Table 2**

<table>
<thead>
<tr>
<th>Process</th>
<th>Parameter</th>
<th>Range and levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unit</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A: Substrate loading</td>
<td>% (w/w)</td>
</tr>
<tr>
<td></td>
<td>B: α-amylose enzyme concentration</td>
<td>U/g</td>
</tr>
<tr>
<td></td>
<td>C: amylloglucosidase enzyme concentration</td>
<td>U/mL</td>
</tr>
<tr>
<td></td>
<td>D: Stroke speed</td>
<td>spm</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A: Yeast concentration</td>
<td>g/L</td>
</tr>
<tr>
<td></td>
<td>B: Reaction temperature</td>
<td>°C</td>
</tr>
<tr>
<td></td>
<td>C: Agitation speed</td>
<td>rpm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(%carbohydrate) = 100% − (%protein + %fat + %ash + %moisture + %water)</th>
</tr>
</thead>
</table>

**Fig. 1.** ANN architecture for the (a) hydrolysis process and (b) fermentation process.

2.9. Fermentation kinetics

The fermentation kinetics of the sorghum grains was determined by using the Gompertz equation modified (Dodić et al., 2012), which is given by the following equation:

\[
Q_p = Q_{p,\text{max}} \times \exp \left\{ \frac{(K_{p,\text{max}} \times \exp (1)}{Q_{p,\text{max}}} \times (t_2 - t) + 1 \right\}
\] (9)

where \(Q_p\) is the predicted ethanol concentration (g/L), \(Q_{p,\text{max}}\) is the maximum concentration of ethanol (g/L), \(K_{p,\text{max}}\) is the maximum rate of ethanol production (g/L/h), \(t_2\) is the lag phase time (h), and \(t\) is the independent variable of time (h). Microsoft Excel Solver is used to determine the coefficient \(Q_{p,\text{max}}, K_{p,\text{max}},\) and \(t_2\) for fermentation condition by non-linear regression of the experimental data.

2.10. Compositional analysis

The compositional content is estimated by determining total nitrogen using the Kjeldahl method (AOAC, 2005a). The protein was calculated from the total nitrogen using the formula \(N \times 6.25\) (Chang, 2010). Soxhlet extraction method (AOAC, 2000) was used to analyse fat content. In addition, the moisture content in dry weight basis may be determined using gravimetric measurement of weight after drying the sample in a hot air oven at 105°C to constant weight. Thus, the ash content was determined by gravimetric measurement of the sample residue after ignition in an oven at 600°C to a constant weight (AOAC 2005b). The fibre was calculated as the loss on ignition of dry residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% solution of sodium hydroxide (AOAC, 2005c). Carbohydrate levels were estimated using the fresh weight-derived data and the following equation (Merrill and Watt, 1973):

\[
\text{(%carbohydrate)} = 100% - (\text{%protein} + \text{%fat} + \text{%ash} + \text{%moisture} + \text{%water})
\]

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>%wt.</td>
<td>73.10</td>
</tr>
<tr>
<td>Proteins</td>
<td>%wt.</td>
<td>10.30</td>
</tr>
<tr>
<td>Ash</td>
<td>%wt.</td>
<td>3.15</td>
</tr>
<tr>
<td>Fibre</td>
<td>%wt.</td>
<td>1.56</td>
</tr>
<tr>
<td>Moisture content</td>
<td>%wt.</td>
<td>10.20</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Composition of sorghum starch

The sorghum starch consists of particles having a diameter within a range of 125–150 μm. It shall be noted that more than 98% of the particles pass through the 150 μm sieve (U.S. Standard No. 100, Certifications ASTM E11-09). The composition of the sorghum starch was determined by chemical analysis and the results are shown in Table 3. It is evident that the sorghum starch is largely composed of carbohydrates (73.10%wt.), making it a desirable substrate to produce bioethanol.

3.2. Modelling and optimization

3.2.1. Modelling of the hydrolysis process using ANN

A total of 29 experimental runs were conducted using four input variables, i.e. substrate loading (10, 15 and 20% (w/v)), α-amylose concentration (90, 100 and 110 U/g), amylloglucosidase concentration (36, 51 and 66 U/mL) and stroke speed (50, 90 and 130 rpm) and one output variable, i.e. reducing sugar concentration. The results obtained from the Box-Behnken experimental design are summarized in Table 4. The experimental data were randomly divided into three sets, whereby the first set was used for training, compris-
ing 70% of the data (21 data points). The second set and third set was used for testing and validation, respectively, whereby each set comprises 15% of the data (four data points).

The reducing sugar concentration was predicted by the ANN model with Levenberg–Marquardt algorithm, consisting of one output and four input neurons. The optimum number of hidden neurons (5 in this case) was found using a heuristic procedure. The neural network architectures and topologies of ANN were selected and tested to predict the reducing sugar concentration. This is due to the fact that it is critical to select an optimum neural network architecture and topology in order to implement ANN successfully. In this sense, determining the optimum number of hidden neurons in the hidden layer was studied by varying the number of neurons from 1 to 10. The estimation and prediction capability of the ANN was assessed based on the following parameters: $R$, $R^2$ and RMSE. The best topology was found to be 4–5–1, which has four inputs, five hidden neurons (optimum) and one output (Fig. 1a) and therefore, this topology was chosen in this study. It can be seen from Table 4 that $R = 0.997$, $R^2 = 0.994$ and RMSE = 1.752 for the whole data set. Since the $R^2$ is greater than 0.80, this indicates that the model has good fit with the experimental data (Joglekar et al., 1987).

The effect of hidden neurons number can be obtained by observing the results of ANN models with other works. The coefficient of determination ($R^2$) in this study is 0.99. It can be compared to Rivera et al. (2010) which found to be lower of 0.97 under the experimental data. Talebnia et al. (2015) performed bioethanol production from steam exploded rapeseed straw and the data of the experimental runs were exploited using artificial neural networks. They found that the coefficient of determination on the optimization of enzymatic hydrolysis from Steam Exploded Straw Rapeseed is 0.979. In addition, the Sankar Vani et al. (2015) carried out application of ANN to predict sugar yields for lignocellulosic biomass during hydrolysis process at various time intervals. The coefficient of determination ($R^2$) of lignocellulosic biomass using ANN is 0.99 which similar to this study.

Indeed, ANN is a massively interconnected network structure consisting of many simple processing elements capable of performing parallel computation for data processing. However, some researchers were using modelling such as RSM and Taguchi to predict the optimization bioethanol production. Instead of coefficient of determination ($R^2$), optimization of glucose formation from Karanja biomass hydrolysis using Taguchi robust by Radhakumari et al. (2014). It has been reported that coefficient of determination ($R^2$) is 0.84 by optimum condition. In addition, Saini et al. (2013) had carried out saccharification of sweet sorghum bagasse used response surface methodology. The result was obtained of 0.98 for coefficient of determination ($R^2$) which lower 1.4% compared to this study.

### 3.2.2. Optimization of the independent variables of the hydrolysis process and sensitivity analysis

In this study, the operating parameters of the hydrolysis process (i.e. substrate loading, $\alpha$-amylase enzyme concentration, amyloglucosidase enzyme concentration and strokes speed) were varied in order to determine the interaction effects of these parameters on the reducing sugar concentration. The ANN model integrated with ACO was used to determine the optimum operating parameters for the hydrolysis process. The optimum substrate loading, $\alpha$-amylase enzyme concentration, amyloglucosidase enzyme concentration and strokes speed is found to be 20% (w/v), 105.5 U/g, 36 U/mL and 50 rpm, respectively. These optimum conditions result in a reducing sugar concentration of 175.94g/L. The model was validated by

<table>
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<th>Amyloglucosidase concentration (U/mL)</th>
<th>Strokes speed (rpm)</th>
<th>Reducing sugar concentration (g/L)</th>
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<th>Experimental value</th>
<th>Predicted value</th>
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Table 4
Box–Behnken experimental design for the hydrolysis process.

$RMSE$, $R$, $R^2$
applying these optimum process parameters to two independent experimental replicates and the average reducing sugar concentration is determined to be 174.29 g/L, which is close to the predicted value. The validation results indicate that the model adequately describes the enzymatic hydrolysis process. The weights of the input and output variables as well as associated biases of the ANN model for the hydrolysis process are presented in Table 5. Fig. 2 shows the level of significance of each input variable involved in the hydrolysis process. The substrate loading, \(\text{a-amylose concentration, amyloglucosidase concentration and strokes speed} \) has a level of significance of 0.3509, 0.2723, 0.2355 and 0.1414, respectively.

### 3.2.3. Modelling of the fermentation process using ANN

The sample with the highest reducing sugar concentration from the hydrolysis process (Section 3.2.1) was then fermented to produce bioethanol. However, there are a number of operating parameters that can restrict fermentation activity, which in turn, affects the ethanol yield. Hence, one can obtain high ethanol yields by optimizing the operating parameters of the fermentation process.

The operating parameter in fermentation process was optimized using ANN model integrated with ACO. Besides, the independent variables chosen for optimization are as follows: (1) yeast concentration (0.5, 1 and 1.5 g/L), (2) reaction temperature (30, 36 and 42 °C) and (3) agitation speed (50, 150 and 250 rpm). The experimental data were randomly divided into three sets, whereby the first set was used for training, comprising 70% of the data (11 data points). The second set and third set was used for testing and validation, respectively, whereby each set comprises 15% of the data (three data points).

The ethanol concentration was predicted by the ANN model with Levenberg-Marquardt algorithm. The model consists of one output layer and three input layers of neurons. The optimum number of hidden neurons (which is 6 in this case) was determined using a heuristic procedure. A number of neural network architectures and topologies of ANN were developed to predict the ethanol concentration. The estimation and prediction capability of the ANN model was evaluated based on the \(R, R^2\) and RMSE values. The best topology is found to be 3–6–1, which has three inputs, six hidden neurons and one output (Fig. 1b). It can be seen from Table 6 that \(R=0.993, R^2=0.987\) and \(\text{RMSE}=2.128\) for the whole data set. Since \(R^2\) is greater than 0.80, this indicates that the model has good fit with the experimental data and it describes more than 80% of the variability in the data (Joglekar et al., 1987). The coefficient of determination \(R^2\) was 0.987 which closed to Betiku and Taiwo (2015), Talebnia et al. (2015) conducted the optimization of simultaneous saccharification and fermentation of straw rapeseed using ANN. The obtained coefficient of determination \(R^2\) is 0.9426. On the other hand, the most important stages in a biological process are modeling and optimization to increase the efficiency of the process. Other researchers such as Wang et al. (2013) have optimized simultaneous saccharification and fermentation of sweet sorghum bagasse using response surface methodology. The coefficient of determination \(R^2\) is 0.92 and it is lower compare to this study. Moreover, Khongsay et al. (2012) observed that the fermentation process of sweet sorghum juice with \(S.\ cevisea\) through an orthogonal array design. The coefficient of determination \(R^2\) of the hydrogenation and fermentation processes is shown in Table 7.

#### 3.2.4. Optimization of the independent variables of the fermentation process and sensitivity analysis

In this study, the operating parameters of the fermentation process (i.e. yeast concentration, reaction temperature and agitation speed) were varied in order to determine the interaction effects of these parameters on the ethanol concentration. The ANN model integrated with ACO was used to determine the optimum operating parameters for the fermentation process. The optimum yeast concentration, reaction temperature and agitation speed is found to be 1.3 g/L, 35.6 °C and 181 rpm, respectively. The ethanol concentration attained at these optimum operating conditions is 82.11 g/L. The ANN model was validated by applying these optimum process parameters to two independent experimental replicates. The average ethanol concentration is 81.52 g/L, which is close to the value predicted by the ANN model. The validation results indicate that the ANN model adequately describes the fermentation process. The weights of the input and output variables as well as associated biases of the ANN model for the fermentation process are summarized in Table 8. Fig. 3 shows the level of significance of the three input variables involved in the fermentation process. The yeast concentration, reaction temperature and agitation speed has a level of significance of 0.4101, 0.3147 and 0.2751, respectively.

#### 3.3. Kinetics of the fermentation process

The sample with the highest reducing sugar concentration obtained from the enzymatic hydrolysis process (Section 3.2.1) was fermented under the optimum conditions (Section 3.2.2) to produce bioethanol. In this study, batch fermentation was used for bioethanol production. The \(S.\ cevisea\) yeast was cultivated in the sorghum hydrolysate solution in order to produce ethanol during the fermentation process. Fig. 4 shows the variation of the reducing sugar and ethanol concentrations with respect to time from the

### Table 5

Weight of the input and output variables of the ANN model for the hydrolysis process.

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Weight input</th>
<th>Weight output</th>
<th>Bias to layer 1</th>
<th>Bias to layer 2</th>
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<td>X2</td>
<td>X3</td>
<td>X4</td>
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<td>-1.5016</td>
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</table>

![Fig. 2. Level of significance of the input variables for the hydrolysis process.](image-url)
fermentation of sorghum starch by *S. cerevisiae*. It can be observed that the glucose is consumed rapidly in the sorghum hydrolysate solution between 12 and 84 h of the fermentation process, which is evident from the declining trend of the reducing sugar concentration. The ethanol yield is determined to be within a range of 0.43–0.47 g ethanol/g glucose, which is equivalent to 85–93% of the theoretical ethanol yield (0.51 g ethanol/g glucose). This result is in good agreement with the results of Moshi et al. (2014) and Kim et al. (2014) who obtained an ethanol yield within a range of 82–84% of the theoretical ethanol yield. The maximum ethanol concentration at 84 h of fermentation is 82.1 g/L, which is significantly higher than the ethanol concentration obtained by Khawla et al. (2014), having a value of 21 g/L. This is due to the fact that *S. cerevisiae* has the greatest activity at 84 h of fermentation.

The kinetic model of fermentation was developed based on the modified Gompertz equation. The purpose of this model is to describe the dynamic change of the ethanol concentration as a function of time during the fermentation process. Therefore, the kinetic model of fermentation was formulated by non-linear regression of the experimental data using Microsoft Excel Solver, as follows:

\[ Q_t = 98.59 \times \exp \left\{ -\exp \left[ \frac{1.47 \times \exp \left( \frac{1}{98.59} \right)}{15.89 - t} \right] + 1 \right\} \]

where \( Q_t \) is the ethanol concentration in grams per litre (g/L) and \( t \) is the fermentation time in hours (h). According to Eq. (11), the maximum ethanol concentration is 98.59 g/L, the maximum reaction rate is 1.47 g/L/h, and the lag time is 15.89 h. The ethanol concentration values predicted by the kinetic model are in good agreement with the measured values, as shown in Fig. 5. The \( R^2 \) value is found to be 0.99.

### 4. Conclusion

In this study, a novel approach is developed to optimize the operating parameters for bioethanol production, in which artificial
neural networks (ANN) are integrated with ant colony optimization (ACO). The model developed in this study is capable of predicting the reducing sugar concentration from enzymatic hydrolysis process and ethanol concentration from the fermentation process. The optimum substrate loading, α-amylase concentration, amyloglucosidase concentration and strokes speed is determined to be 20% (w/v), 109.5 U/g, 36 U/mL and 50 rpm, respectively, which gives a reducing sugar concentration of 175.94 g/L. The predicted reducing sugar concentration is close to the average value obtained from experiments, which is 174.29 g/L. The validation results indicate that the model has good fit with the experimental data. The hydrolysate obtained from optimum operating parameters during enzymatic hydrolysis is used in the fermentation process. The predicted (calculated) ethanol concentration from the fermentation process by S. cerevisiae is found to be 82.11 g/L, which is close to the experimental value of 81.52 g/L. Based on the results, it can be concluded that the ANN model developed in this study is useful to predict the optimum operating parameters which will maximize the reducing sugar concentration and ethanol concentration from hydrolysis and fermentation, respectively. This model is indeed beneficial for bioethanol production since it can help reduce the time, cost and effort associated with experimental techniques.

![Figure 3](image3.png)  
**Fig. 3.** Level of significance of the process.

![Figure 4](image4.png)  
**Fig. 4.** Variation of ethanol and reducing sugar concentrations during batch fermentation by S. cerevisiae yeast.

![Figure 5](image5.png)  
**Fig. 5.** Fitting of the modified Gompertz model to the experimental data of the fermentation process.

### Acknowledgements

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