Using an artificial neural network to predict the optimal conditions for enzymatic hydrolysis of apple pomace

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Received: 1 September 2016 / Accepted: 23 January 2017 / Published online: 8 June 2017
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Abstract The enzymatic degradation of lignocellulosic biomass such as apple pomace is a complex process influenced by a number of hydrolysis conditions. Predicting optimal conditions, including enzyme and substrate concentration, temperature and pH can improve conversion efficiency. In this study, the production of sugar monomers from apple pomace using commercial enzyme preparations, Celluclast 1.5L, Viscozyme L and Novozyme 188 was investigated. A limited number of experiments were carried out and then analysed using an artificial neural network (ANN) to model the enzymatic hydrolysis process. The ANN was used to simulate the enzymatic hydrolysis process for a range of input variables and the optimal conditions were successfully selected as was indicated by the R^2 value of 0.99 and a small MSE value. The inputs for the ANN were substrate loading, enzyme loading, temperature, initial pH and a combination of these parameters, while release profiles of glucose and reducing sugars were the outputs. Enzyme loadings of 0.5 and 0.2 mg/g substrate and a substrate loading of 30% were optimal for glucose and reducing sugar release from apple pomace, respectively, resulting in concentrations of 6.5 g/L glucose and 28.9 g/L reducing sugars. Apple pomace hydrolysis can be

successfully carried out based on the predicted optimal conditions from the ANN.

Keywords ANN · Apple pomace · Enzyme loading · Modelling · Substrate loading

Introduction

The production of biofuels from lignocellulosic wastes has received attention from researchers around the world (Dashtban et al. 2009; Del Rio et al. 2012; Garcia-Aparicio et al. 2011). Biofuels can reduce the world's dependency on non-renewable fossil fuels, which also causes environmental pollution, global warming and conflict in some regions of the world (Dashtban et al. 2009; Garcia-Aparicio et al. 2011; Himmel et al. 2007; Merino and Cherry 2007). Lignocellulosic biomass is heterogeneous and insoluble and requires multiple enzymes working in synergy for its degradation (Dashtban et al. 2009; Teeri 1997). Apple pomace, a waste product from the apple juice industry, is a potential lignocellulosic feedstock for the production of biofuels and biorefinery chemicals (Gama et al. 2015). Apple pomace is composed mainly of cellulose, pectin and hemicellulose, which can be converted to sugar monomers and other value-added products using multiple enzymes working in synergy (Bhushan et al. 2008; Gama et al. 2015; Joshi and Attri 2006; Parmar and Rupasinghe 2013). Enzymes required for the degradation of the cellulose component include cellobiohydrolases, endo-glucanases and β -glucosidases (Teeri 1997). Pectin degradation requires enzymes such as methyl esterase, acetyl esterase, pectate lyase, pectin lyase, endo-polygalacturonase, exo-polygalacturonase, arabinase and galactanase (Gama et al. 2015; Voragen et al. 2009), while degradation of

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hemicellulose requires enzymes like endo-xylanases, β -xylosidases, xyloglucanases, as well as possible debranching enzymes. Commercial enzyme cocktails are available which act on lignocellulosics, with different cocktails optimised for different substrates (Gama et al. 2015; Parmar and Rupasinghe 2013).

The commercial production of biofuels is hampered by enzyme and pretreatment costs with the saccharification step being the most rate limiting step (Lynd et al. 2008; O'Dwyer et al. 2008). Optimising the process conditions for conversion of lignocellulosic substrates to sugar monomers will improve the economic feasibility of commercial biofuel production. Reliable kinetic models, therefore, need to be developed to facilitate selection of optimal process conditions. Enzyme kinetics is influenced by the interplay of a number of factors, whose effect should be assessed together. The main parameters that affect enzymatic hydrolysis of lignocellulose biomass are enzyme and substrate concentrations and hydrolysis conditions such pH, temperature and time (Bansal et al. 2009; Gan et al. 2003; Gupta et al. 2012). However, taking into account all these factors at once is a complex task, especially for heterogeneous substrates like apple pomace, a waste product from the apple juice industry. These factors make the modelling of enzyme kinetics in these complex substrates difficult and poorly understood (Al-Zuhair 2008; Andric et al. 2010b; Kadam et al. 2004; Sousa et al. 2011). Most models are able to explain hydrolysis during the initial stages of hydrolysis, but not at the later stages, where the rate of reaction slows down due to end-product inhibition, mass transfer limitations, inactivation of the enzymes and unproductive adsorption of enzymes on the substrate (Bommarius et al. 2008; Sarkar and Etters 2004; Zhang et al. 1999). A number of kinetic models to predict enzymatic hydrolysis of biomass have been developed and reviewed in the literature which include Michaelis–Menten models; empirical models; mechanistic models; models accounting for adsorption; models for soluble substrates; functionally based and structurally based models (Bansal et al. 2009; Sousa et al. 2011; Wang et al. 2011).

Empirical models use a small number of parameters to understand the initial rate of hydrolysis and how it is affected by different conditions e.g. temperature, pH, enzyme, substrate properties and time (Bansal et al. 2009). Empirical models are easy to understand due to the small number of parameters that are considered and are therefore suitable for industrial application (Bansal et al. 2009; Holtzappple et al. 1984). Empirical models have some drawbacks, e.g. they have limited use outside the conditions under which they are developed and do not fully describe the reaction mechanisms involved in the enzyme-substrate interaction (Wang et al. 2011). Large empirical data in complex systems involving many parameters can be modelled using an artificial neural network (ANN), which is a computational mathematical network, which has been used in fields like engineering,

medicine and food industry (Bhotmange and Shastri 2011; Kalogirou 2000; O'Dwyer et al. 2008; Wang et al. 2011). ANN has also been used in modelling lignocellulose biomass hydrolysis—for example in the simultaneous saccharification and fermentation (SSF) of sugarcane bagasse using temperature, initial pH and fermentation time data (Ezhumalai and Thangavelu 2010; Sasikumar and Viruthagiri 2010). O'Dwyer et al. (2008) used ANN to successfully predict the effect of acetyl and lignin contents and crystallinity indices on the digestibility of poplar wood. ANN was also successfully used to model the effect of pretreatment conditions (temperature, time, solid content and sulphuric acid concentration) on glucose production from biomass (Sousa et al. 2011). Glucose yield from sugarcane bagasse using different loadings of cellulase and β -glucosidase was predicted by Rivera et al. (2010). Zhang et al. (2010) used ANN to better predict glucose concentration by considering three hydrolysis parameters; substrate concentration, cellulase concentration and hydrolysis time.

The advantage of ANN is that it allows for rational optimisation of variables and processes using reduced data in situations where there is insufficient detailed knowledge of the underlying process and formulation of a reaction mechanism is not possible as compared to traditional empirical models (Bhotmange and Shastri 2011; Nodeh 2012). Complex interactions of variables can be evaluated at the same time as more data can be analysed at a time by ANNs (Bhotmange and Shastri 2011; Sousa et al. 2011). ANNs offer enormous potential in complex systems such as the bioconversion of lignocellulose (Nodeh 2012; O'Dwyer et al. 2008; Sasikumar and Viruthagiri 2010).

The aim of this study was to develop an artificial neural network to predict optimal hydrolysis conditions for glucose and reducing sugar production from apple pomace using commercial enzyme preparations, Celluclast 1.5L, Viscozyme L and Novozyme 188. The focus was on the main factors that influence apple pomace hydrolysis such as change in substrate loading, enzyme loading, temperature and pH over time in a batch reactor system. It was envisaged that these parameters were important for an industrial bioreactor process. Predicting optimal conditions for apple pomace hydrolysis successfully is of paramount importance for the design of efficient and cost-effective hydrolysis of apple pomace which will reduce capital and operational costs for the production of biofuels and biorefinery chemicals.

Materials and methods

Substrate and enzymes

Apple pomace was prepared from Golden delicious apples that were obtained from a local supermarket (His Majesty's

Fruit and Vegetables, Grahamstown, South Africa). Apple juice was separated from the pomace by means of a kitchen juicer. The extracted pomace was then homogenised in a blender. Free sugars in the apple pomace were washed off using distilled water and excess water squeezed out using a muslin cloth. The prepared apple pomace was stored at -20°C until use. Sodium azide (NaN_3) at a final concentration of 0.03% (w/v) was added as a microbial preservative to buffers and apple pomace. The composition of apple pomace was as follows: glucose 22.3%, arabinose 12.5%, galactose 5.1%, xylose 1.1%, galacturonic acid $\sim 38\%$ and lignin 19.8%, as previously reported (Gama et al. 2015).

Three commercial enzyme preparations were used in this study, Viscozyme L (from *Aspergillus aculeatus*, hemicellulase, arabinase, β -glucanase, cellulase and xylanase activities), Celluclast 1.5L (from *Trichoderma reesei* ATCC 26921, a cellulase preparation) and Novozyme 188 (a commercial *Aspergillus niger*, β -glucosidase preparation) all obtained from Sigma (South Africa). Viscozyme L and Celluclast 1.5L were used on a ratio of 50:50 and Novozyme 188 as 10% of the total protein (Gama et al. 2015).

Sugar analysis

The amount of reducing sugars released from apple pomace after enzymatic hydrolysis were determined as glucose equivalents using a modified 3,5-dinitrosalicylic acid (DNS) assay method (Miller 1959). The DNS assay was performed as was described by Beukes et al. (2008) using a glucose standard curve. Glucose concentration was estimated using a Megazyme commercial kit (Megazyme, Ireland) according to the manufacturer's instruction manual. The carbohydrates content of apple pomace was determined in our previous work (Gama et al. 2015).

Enzymatic hydrolysis for the generation of artificial neural network experimental data

Experimental data input for the artificial neural network was generated using varying substrate loading, enzyme loading, initial pH, temperature and a combination of these parameters. Bioreactors were operated in batch mode using 1 L Schott bottles with mixing of the reaction taking place with compressed air, for a period of 100 h. Aliquots of 5 mL were collected at various time intervals and reactions in the samples stopped by heating at 100°C for 5 min. The concentration and yield of the liberated sugars were determined as described above ("Sugar analysis").

The effect of different apple pomace loadings on enzymatic hydrolysis was determined using final substrate loadings of 5, 10, 20 and 30% (wet, w/v) in deionised water

which corresponded to 0.5, 1, 2, and 3% dry weight (dry weight was determined using freeze-drying method). The high viscosity at these loadings precluded the use of higher loadings as mixing became impossible.

Hydrolysis was performed using Viscozyme L—Celluclast 1.5L (50:50) ($0.5\ \mu\text{L/mL}$, $0.038\ \text{mg/mL}$ final for each) and Novozyme 188 ($0.05\ \mu\text{L/mL}$, $0.0024\ \text{mg/mL}$). Selection of this ratio was based on optimisation studies that we had carried out. The effect of initial pH on hydrolysis was investigated using unbuffered deionised water and citrate buffer at pH 4.0 and pH 5.0 (0.05 M). The effect of temperature on hydrolysis was determined by using room temperature ($22\text{--}26^{\circ}\text{C}$), 28 and 37°C . The conditions used were based on range of optimum activities of the enzyme mixtures and also the need to employ cost-effective methods (Gama et al. 2015).

Finally, the effect of total enzyme loading was evaluated using Viscozyme L and Celluclast 1.5L (50:50) loadings of 0.12, 0.2, 0.4 and 0.6 mg protein/g apple pomace (wet w) and 10% protein loading of Novozyme 188 in deionised water. Apple pomace, 20% (wet, w/v) was used as substrate. The interactive effect of various hydrolysis conditions was evaluated using various combinations of substrate loading, enzyme loading, temperature and pH. The rest of the procedures were as described above.

Construction of artificial neural network

The experimental data generated from "Enzymatic hydrolysis for the generation of artificial neural network experimental data" were used for the supervised learning and training of the various neural networks using Matlab. There were 27 different input vectors, each replicated 3 times, giving 81 columns in the data matrix. Input patterns were 4-dimensional and targets were 6-dimensional each for glucose and reducing sugars. Separate ANNs were constructed for glucose and reducing sugars. There were 6 input patterns for enzyme loading, substrate loading, initial pH, temperature values and a combination of these factors values and 6 target/output variables of glucose/reducing sugars concentration values for 1, 6, 24, 50, 72 and 100 h hydrolysis times.

Each input pattern was a column vector of the form:

$$\begin{bmatrix} p1 \\ p2 \\ p3 \\ p4 \\ p5 \\ p6 \end{bmatrix}$$

where $p1$ is the lower temperature (22 for room temperature, 28 and 37°C), $p2$ is the upper temperature (26 for room temperature, 28 and 37°C), $p3$ is the lower pH, $p4$ is

the upper pH, p_5 is the enzyme concentration, p_6 is the substrate concentration.

Each target was a 'release profile' of the form:

$$\begin{bmatrix} t_1 \\ t_2 \\ t_3 \\ t_4 \\ t_5 \\ t_6 \end{bmatrix}$$

where t_1 is the amount of glucose/reducing sugar after 1 h, t_2 is the amount of glucose/reducing sugar after 6 h, t_3 is the amount of glucose/reducing sugar after 24 h, t_4 is the amount of glucose/reducing sugar after 50 h, t_5 is the amount of glucose/reducing sugar after 72 h, t_6 is the amount of glucose/reducing sugar after 100 h.

These readings were recorded in triplicate and there were 27 columns in the spreadsheet with the readings concatenated into an 18×1 vector.

The triplicate outputs for sugar release were perturbed, the first of the input values was kept and the remaining two values were perturbed by a small amount (0.01). As a result 81 different input patterns and associated targets were obtained, which was enough for the ANN to perform reasonably. Multiple layer neural networks with interconnected neurons arranged in layers that are input, hidden and output layers were constructed. Each layer was interconnected by weights (w) and biases (b) which were adjustable, enabling it to model non-linear processes. After experimentation with various architectures, an ANN composed of 20, 20 and 6 neurons in the first, second and third layer, respectively and one output layer with one neuron was chosen for both glucose and reducing sugars. The three transfer functions were *tansig*, *tansig* and *purelin*.

The data set was portioned into train, test, validation sets (2/3, 1/6, 1/6). Training of the ANN was performed using the scaled conjugate gradient (*trainscg*) training algorithm, first over 1000 epochs and then 500 epochs to check the effect on correlation coefficient, R^2 and mean square error (MSE). After observing that ANN was simulating accurately on the test points, the ANN was then trained on all data. Using a nested loop, the trained neural network was used to simulate on a range of input conditions and the corresponding outputs were stored. Then the input vector which produced an optimal output was found using the *max* function. A Matlab function, *max*, was used to sort glucose and reducing sugar outputs and their corresponding input patterns in decreasing order of sugar concentration. The maximum sugar concentrations were plotted to show optimal conditions for hydrolysis. In this study, maximum sugar release when temperature was fixed at room temperature and pH as unbuffered and varying enzyme loadings and substrate loadings were plotted.

Results and discussion

Effect of substrate loading

The effect of various substrate loadings on enzymatic hydrolysis of apple pomace was determined. The amount of glucose and reducing sugars released are shown in Fig. 1. An increase in substrate loading resulted in an increase in the amount of sugars released. The release of sugars increased linearly at the beginning of the reaction, but the rate decreased with time, similar to results by other authors (Andric et al. 2010c; Bansal et al. 2009; Zhang et al. 1999). The rapid initial release of sugars can be attributed to high synergy between enzymes as the substrate was modified as well as the availability of easily hydrolysable sites on the substrate. As the reaction proceeds, hydrolysis slows down as there will be changes in substrate structure, with easily hydrolysable substrate getting depleted, enzymes may become adsorbed on residual substrate and lignin, enzymes may become deactivated, end-product inhibition may occur as well as mass transfer limitations, especially at high substrate loadings (Bommarius et al. 2008; Sarkar and Etters 2004; Zhang et al.

Fig. 1 Concentration of sugars released at different time intervals for the batch reactor mixed with compressed air at room temperature with different substrate loadings. **a** (Glucose) and **b** (reducing sugars). Data points are presented as mean values $\pm$ SD ($n = 3$)

1999). Glucose was released rapidly at the beginning of hydrolysis at low substrate loading (Lu et al. 2010). The fast rate of glucose release may be due to the removal/hydrolysis of the first layer of cellulose (Gan et al. 2003). Other sugars like arabinose, galactose as well as galacturonic acid might have been released first before glucose, contributing to the difference in pattern between reducing sugars and glucose (Gama et al. 2015). The results for reducing sugars showed that at higher substrate loadings, the initial rate of reaction was faster, hence the higher production of sugars in agreement with Gan et al. (2003). Shorter hydrolysis times were required for low substrate loadings as compared to longer hydrolysis times for high substrate loading, which was in agreement to reported work on CMC and corn stover using cellulase (Al-Zuhair 2008; Lu et al. 2010). This may be due to high initial viscosity at high substrate loadings which poses mass transfer limitations, resulting in a low conversion efficiency (Ioelovich and Morag 2012; Lu et al. 2010; Mussatto et al. 2008; Sarkar and Etters 2004). End-product inhibition on cellulases by cellobiose was avoided by supplementing β -glucosidase using Novozyme 188 (Teeri 1997). High substrate loading results in increased sugar concentration, which assisted in lowering capital operational costs associated with reduced reactor volume, reduced energy costs and ethanol separation (Hodge et al. 2009; Lu et al. 2010; Zhang et al. 2010). However, the limitation of high solids is that more energy is required for mixing and there is a build-up of residual solids. Substrate loadings beyond 30% (wet w/v) could not be mixed using compressed air.

Effect of enzyme loading

The effect of enzyme loadings on enzymatic hydrolysis of apple pomace was established. The amount of glucose and reducing sugars released are shown in Fig. 2. There was an increase in sugar concentration with an increase in enzyme loading and time. Sugar release was high at the initial stages of hydrolysis and began to plateau after 50 h for glucose and 24 h for reducing sugars. The initial reaction rate was faster and greater at higher enzyme loading and a higher amount of sugars was released as compared to low enzyme loadings. This may be due to high enzyme to substrate ratio at high enzyme loading resulting in higher conversion efficiency (Rosgaard et al. 2007). At low enzyme loadings, all hydrolysis sites on the substrate are not yet saturated, and the degree of synergy is normally low. Almost similar amounts of sugars were released at enzyme loadings of 0.4 and 0.6 mg enzyme/g substrate, which may be due to the enzymes having reached the saturation point. There may also be enzyme jamming beyond saturation point as enzyme molecules interfere with each

Fig. 2 Concentration of sugars released at different time intervals for the batch reactor mixed with compressed air at room temperature with 20% (wet, w/v) apple pomace. **a** (Glucose) and **b** (reducing sugars). Data points are presented as mean values $\pm$ SD ($n = 3$)

other (Andric et al. 2010a; Bommarius et al. 2008; Rosgaard et al. 2007).

It has been reported by some researchers that enzyme concentration had a more significant impact than substrate concentration on the release of sugars, which was in agreement with results obtained in this study (Mussatto et al. 2008; Rosgaard et al. 2007; Vasquez et al. 2007).

Influence of temperature

The effect of temperature on hydrolysis was investigated at room temperature, 28 and 37 °C. The amount of glucose and reducing sugars released by each treatment were as shown in Fig. 3a, b, respectively. At high incubation temperatures the initial reaction rate was high compared with low temperatures. However, the amount of sugars released after 100 h was almost the same. The amount of glucose released was 4, 3.9 and 4.2 g/L and reducing sugars, 18, 17.7 and 16.8 g/L at 37, 28 °C and room temperature, respectively after 100 h hydrolysis. Previous results indicated that both Celluclast 1.5L and Viscozyme L had temperature optima of 50 °C, but Celluclast 1.5L maintained a high activity even at 20 °C and Viscozyme L

at temperatures above 25 °C (results not shown). High temperatures were not used in this study in order to reduce energy input, while also maintaining conditions suitable for simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP), which often use temperatures between 30 and 37 °C (Andric et al. 2010a; Van Dyk and Pletschke 2012). Using low temperatures lowers energy costs for an industrial process as less or no heating will be required.

Influence of pH

The effect of initial pH on hydrolysis was investigated using an unbuffered system, pH 4.0 and pH 5.0. Figure 4a, b show the amount of glucose and reducing sugars (including galacturonic acid) released overtime. An unbuffered system was chosen so as to reduce the cost of the process. Surprisingly, the use of an unbuffered system resulted in the release of higher amounts of sugars than the buffered system. At pH 5.0 more glucose was released than

pH 4.0, but it was the opposite for reducing sugars. The amount of glucose released was 3.4, 4.1 and 4.2 g/L and reducing sugars, 15, 13.8 and 16.8 g/L using citrate buffer at pH 4.0, pH 5.0 and an unbuffered system, respectively after 100 h hydrolysis. The initial pH for the unbuffered system was around pH 5.0 and dropped to around pH 3.2 after 100 h. The results indicated that the initial pH as well as change in pH as the reaction progresses was important for the hydrolysis of apple pomace. The change in pH as the reaction progress may result in some enzymes in the commercial mixtures being inhibited, while others may display enhanced or stimulated at different pH values. The decrease in pH could be mainly as a result of galacturonic acid release (Gama et al. 2015). Our previous results indicated that the optimum pH for Celluclast 1.5L and Viscozyme L was between pH 4.0 and pH 5.0, but both maintained high activities between pH 3.0 and pH 6.0 due to the different optima values for the different enzymes present in the commercial enzyme cocktails (Gama et al. 2015). It has also been demonstrated in our previous work

Fig. 3 The amount of sugars released at different time intervals for the batch reactor mixed with compressed air at different temperatures with 20% (wet, w/v) apple pomace and enzyme loading of 0.2 mg/g substrate. Unbuffered deionised water was used in the reactions. **a** (Glucose) and **b** (reducing sugars). Data points are presented as mean values $\pm$ SD ($n = 3$)

Fig. 4 The amount of sugars released at different time intervals for the batch reactor mixed with compressed air at different initial pH conditions with 20% (wet, w/v) apple pomace and enzyme loading of 0.2 mg/g substrate. Hydrolysis was performed at room temperature. **a** (Glucose) and **b** (reducing sugars). Data points are presented as mean values $\pm$ SD ($n = 3$)

that the pectin and hemicellulose components of apple pomace are hydrolysed first, thereby opening up the structure for cellulose hydrolysis (Gama 2014; Gama et al. 2015). Using an unbuffered system in industrial applications could be very advantageous and lead to reduced costs.

Interactive effect of different hydrolysis parameters

The effect of a combination of different parameters on enzymatic hydrolysis of apple pomace was investigated, including some substrate and enzyme concentrations as well as pH and temperature values which were not used in the experiments above (“Effect of substrate loading”, “Effect of enzyme loading”, “Influence of temperature”, “Influence of pH”). The amount of sugars released over time was as shown in Fig. 5. The results indicated that substrate and enzyme loading were the most important parameters for apple pomace hydrolysis. A high amount of sugars were released when the reactions were performed at room temperature in an unbuffered system using high substrate and enzyme loadings (up to the optimum). The results indicated that hydrolysis of apple pomace was influenced by the interaction of different parameters, though to a different magnitude. The obtained results were similar to what was reported by Vasquez et al. (2007) on hydrolysis of sugarcane bagasse: that enzyme loading had the highest effect followed by temperature, when they considered the effect of pH, percentage solids, enzyme loading and temperature. There was less significant effect on hydrolysis as a result of interaction of factors and pH had less effect compared with other variables in the range considered. The difference with the results obtained in this work may be related to different substrates and the range of parameters used. In the work by Parmar and Rupasinghe (2013), pH, temperature, time and enzyme dosage were shown to be significant for the release of sugars from apple pomace using a combination of Celluclast 1.5L, Pectinex 3XL and Novozyme 188. Though the same substrate was used, the enzymes used were not all the same and the range of values for the parameters used were also different.

ANN

The ANN for both glucose and reducing sugars were successfully trained. The input parameters (e.g. temperature, pH, enzyme and substrate loadings and a combination of these parameters) that provided the most valuable information for apple pomace hydrolysis were selected for the development of the ANN model. The selection of the most appropriate parameters for ANN modelling is considered of paramount importance for prediction of the hydrolysis process (Puig-Arnau et al. 2013; Rivera et al. 2010). The constructed ANN was assessed for its accuracy for

Fig. 5 The amount of sugars released at different time intervals for the batch reactor mixed with compressed air a combination of parameters. Apple pomace was used as the substrate. **a** (Glucose) and **b** (reducing sugars). UB unbuffered, RT room temperature, S substrate (% wet w/v), E enzyme (mg/g substrate). Data points are presented as mean values $\pm$ SD ($n = 3$)

generalisation and predictive ability using R^2 value and MSE values for the outputs. The ANN fitted values during training were compared with the experimental data and the fit accuracy was high as indicated by the R^2 value of 0.99 and small MSE value for reducing sugar concentrations on all the data. The model was successful in predicting sugar release from apple pomace. The values for ANN prediction for sugar concentration were very close to the experimental values (Fig. 6), further confirming the successful training and predictive ability of the developed ANN. It has been reported in literature that ANNs are flexible as new data can be added anytime giving fitting (Bhotmange and Shastri 2011; O'Dwyer et al. 2008; Rivera et al. 2010; Sousa et al. 2011; Wang et al. 2011).

After predicting values accurately, ANN model was used to determine the optimum hydrolysis conditions for apple pomace by varying substrate and enzyme loadings and fixing temperature at room temperature and pH as unbuffered. These conditions were selected based on the results from the experimental data (“Influence of temperature”, “Influence of pH”). The sugar concentration release profiles were sorted according to sugar

Fig. 6 Experimental data output values compared with ANN predicted values for glucose (a) and reducing sugars (b). ANN predicted values (○) and experimental values (+). Conc concentration

concentrations using the Matlab *max* function. The maximum glucose and reducing sugar concentrations profiles were then used to generate surface area plots (Fig. 7). The optimal substrate loading for both glucose and reducing sugars release was 30% (wet w/v), but enzyme loading was different, 0.5 mg/g substrate for glucose release and 0.2 mg/g substrate for reducing sugars. The maximum glucose concentration was 6.5 g/L and reducing sugars 28.9 g/L after 100 h. An increase in substrate loading resulted in an increase in sugar release, however substrate loadings above 30% (wet w/v) could not be used in this study due to difficulties in mixing (“Effect of substrate loading”). The maximum enzyme loading did not result in maximum sugar concentrations. Enzyme loadings below and above the optima resulted in lower sugar concentrations (“Effect of enzyme loading”). Minimum enzyme loading is important for cost-effective biofuel production (Lynd et al. 2008; Rivera et al. 2010; Zhang et al. 2010).

Fig. 7 Response surface plots showing maximum glucose (a) and reducing sugars (b) released under different substrate and enzyme concentrations. Substrate concentration (% wet w/v), enzyme loading (mg/g substrate), gluc (glucose) and RS (reducing sugar)

The enzyme loading used for industrial application will depend on the targeted product. The reducing sugars component includes sugars such as arabinose, galactose and xylose as well as galacturonic acid (Gama et al. 2015). The released sugars together with galacturonic acid can be used for biofuel production as well as production of biorefinery chemicals e.g. xylitol, sorbitol (Boluda-Aguilar et al. 2010; Bhushan et al. 2008; Van Dyk et al. 2013). Substrate and enzyme loadings had an interactive effect on the release of sugars and were the most influential parameters for apple pomace hydrolysis. The results from this study indicate that ANN can be used to simulate apple pomace hydrolysis and predict sugar release. Most of the developed models by researchers were based on cellulases action on cellulose or pretreated substrates to produce glucose and cellobiose and have not been adequate to explain lignocellulose hydrolysis (Bansal et al. 2009; Hodge et al. 2009; Sousa et al. 2011).

It has been reported that ANN needs fewer experiments, is not affected by experimental design and is flexible as it can predict values from the unseen/unmeasured data (Rivera et al. 2010; Wang et al. 2011; Zhang et al. 2010). However, the limitation of ANN is that it cannot explain

the interactive mechanism between the enzyme and the substrate (Brown et al. 2010; Sousa et al. 2011; Wang et al. 2011). However, this underlying mechanism is approximated by the ANN.

Conclusions

A number of parameters influence enzymatic hydrolysis of apple pomace such as temperature, initial pH, enzyme loading, substrate loading and time of hydrolysis. ANN model was successfully developed using the most significant parameters. ANN managed to correctly simulate and predict non-linear data such as apple pomace hydrolysis and can be used for determining the optimal hydrolysis conditions. Enzyme loadings of 0.5 and 0.2 mg/g substrate and a substrate loading of 30% were optimal for glucose and reducing sugar release from apple pomace, respectively. The developed ANN can be used for process optimisation and control. The ability to predict optimal sugar concentrations is important for the design of a cost-effective process for apple pomace hydrolysis. The results of this study indicate that other hydrolysis conditions can be successfully modelled using the ANN. It can therefore be concluded that ANN can be successfully applied in complex systems like lignocellulose hydrolysis.

Acknowledgements The authors gratefully acknowledge the funding received from the Water Research Commission (WRC, K5/2009.1) of South Africa.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

References

- Al-Zuhair S (2008) The effect of crystallinity of cellulose on the rate of reducing sugars production by heterogeneous enzymatic hydrolysis. *Bioresour Technol* 99:4078–4085
- Andric P, Meyer AS, Jensen PA, Dam-Johansen K (2010a) Effect and modeling of glucose inhibition and in situ glucose removal during enzymatic hydrolysis of pretreated wheat straw. *Appl Biochem Biotechnol* 160:280–297
- Andric P, Meyer AS, Jensen PA, Dam-Johansen K (2010b) Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes. *Biotechnol Adv* 28(3):308–324
- Andric P, Meyer AS, Jensen PA, Dam-Johansen K (2010c) Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis II. Quantification of inhibition and suitability of membrane reactors. *Biotechnol Adv* 28:407–425
- Bansal P, Hall M, Realf MJ, Lee JH, Bommarius AS (2009) Modelling cellulase kinetics on lignocellulosic substrates. *Biotechnol Adv* 27:833–848
- Beukes N, Chan H, Doi RH, Pletschke BI (2008) Synergistic associations between *Clostridium cellulovorans* enzymes XynA, ManA and EngE against sugarcane bagasse. *Enzyme Microb Technol* 42:492–498
- Bhotmange M, Shastri P (2011) Application of artificial neural networks to food and fermentation technology. In: Suzuki K (ed) *Artificial neural networks—industrial and control engineering applications*. InTech, Rijeka, pp 201–222. ISBN 978-953-307-220-3
- Bhushan S, Kalia K, Sharma M, Singh B, Ahuja PS (2008) Processing of apple pomace for bioactive molecules. *Crit Rev Biotechnol* 28:285–296
- Boluda-Aguilar M, Garcia-Vidal L, Gonzalez-Castaneda FDP, Lopez-Gomez A (2010) Mandarin peel wastes pretreatment with steam explosion for bioethanol production. *Bioresour Technol* 101:3506–3513
- Bommarius AS, Katona A, Cheben SE, Patel AS, Ragauskas AJ, Knudson K, Pu Y (2008) Cellulase kinetics as a function of cellulose pretreatment. *Metab Eng* 10:370–381
- Brown RF, Agbogbo FK, Holtzaple MT (2010) Comparison of mechanistic models in the initial rate enzymatic hydrolysis of AFEX-treated wheat straw. *Biotechnol Biofuels* 3:6
- Dashtban M, Schraft H, Qin W (2009) Fungal bioconversion of lignocellulosic residues; opportunities and perspectives. *Int J Biol Sci* 5(6):578–595
- Del Rio LF, Chandra RP, Saddler JN (2012) Fibre size does not appear to influence the ease of enzymatic hydrolysis of organosolv-pretreated softwoods. *Bioresour Technol* 107:235–242
- Ezhumalai S, Thangavelu V (2010) Kinetic and optimization studies on the bioconversion of lignocellulosic material into ethanol. *BioRes* 5(3):1879–1894
- Gama R (2014) A lignocellulolytic system for fruit waste degradation: commercial enzyme mixture synergy and bioreactor design. Ph.D. thesis, Rhodes University, South Africa
- Gama R, Van Dyk SJ, Pletschke BI (2015) Optimization of enzymatic hydrolysis of apple pomace for the production of biofuel and biorefinery chemicals using commercial enzymes. *3Biotech*. doi:10.1007/s13205-015-0312-7
- Gan Q, Allen SJ, Taylor G (2003) Kinetic dynamics in heterogeneous enzymatic hydrolysis of cellulose: an overview, an experimental study and mathematical modelling. *Process Biochem* 38:1003–1018
- Garcia-Aparicio M, Trollope K, Tyhoda L, Diedericks D, Gorgens J (2011) Evaluation of triticale bran as raw material for bioethanol production. *Fuel* 90:1638–1644
- Gupta R, Kumar S, Gomes J, Kuhad RC (2012) Kinetic study and fed-batch enzymatic saccharification of pretreated substrate and subsequent fermentation to ethanol. *Biotechnol Biofuels* 5:16
- Himmel ME, Ding S-Y, Johnson D, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 315:804–807
- Hodge DB, Karim MN, Schell DJ, McMillan JD (2009) Model-based fed-batch for high-solids enzymatic cellulose hydrolysis. *Appl Biochem Biotechnol* 152:88–107
- Holtzaple MT, Caram HS, Humphrey AE (1984) A comparison of two empirical models for the enzymatic hydrolysis of pretreated poplar wood. *Biotechnol Bioeng* 26:936–941
- Ioelovich M, Morag E (2012) Study of enzymatic hydrolysis of pretreated biomass at increased solids loading. *BioRes* 7(4):4672–4682
- Joshi VK, Attri D (2006) Solid state fermentation of apple pomace for the production of value added products. *Nat Prod Rad* 5(4):289–296
- Kadam KL, Rydholm EC, McMillan JD (2004) Development and validation of a kinetic model for enzymatic saccharification of lignocellulosic biomass. *Biotechnol Prog* 20:698–705

- Kalogirou SA (2000) Applications of artificial neural-networks for energy systems. *Appl Energ* 67:17–35
- Lu Y, Wang Y, Xu G, Chu J, Zhuang Y, Zhang S (2010) Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. *Appl Biochem Biotechnol* 160:360–369
- Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R (2008) How biotechnology can transform biofuels. *Nat Biotechnol* 26:169–172
- Merino ST, Cherry J (2007) Progress and challenges in enzyme development for biomass utilisation. *Adv Biochem Eng Biotechnol* 108:95–120
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
- Mussatto SI, Dragone G, Fernandes M, Milagres AMF, Roberto IC (2008) The effect of agitation speed, enzyme loading and substrate concentration on enzymatic hydrolysis of cellulose from brewer's spent grain. *Cellulose* 15:711–721
- Nodeh AA (2012) Design of a neural network for a concentrated acid hydrolysis of biomass in bioethanol production as a clean energy. International conference on agriculture, chemical and environmental sciences (ICACES'2012), Dubai (UAE), Oct. 6–7, pp 143–146
- O'Dwyer JP, Zhu L, Granda CB, Chang VS, Holtzaple MT (2008) Neural network prediction of biomass digestibility based on structural features. *Biotechnol Prog* 24:283–292
- Parmar I, Rupasinghe HPV (2013) Bio-conversion of apple pomace into ethanol and acetic acid: enzymatic hydrolysis and fermentation. *Bioresour Technol* 130:613–620
- Puig-Arnavat M, Hernandez A, Bruno JC, Coronas A (2013) Artificial neural network models for biomass gasification in fluidized bed gasifiers. *Biomass Bioenerg* 49:279–289
- Rivera EC, Rabelo SC, Garcia DR, Filho RMC, Costa AC (2010) Enzymatic hydrolysis of sugarcane bagasse for bioethanol production: determining optimal enzyme loading using neural networks. *J Chem Technol Biotechnol* 85:983–992
- Rosgaard L, Andric P, Dam-Johansen K, Pedersen S, Meyer AS (2007) Effect of substrate loading on enzymatic hydrolysis and viscosity of pretreated barley straw. *Appl Biochem Biotechnol* 143:27–40
- Sarkar AK, Etters JN (2004) Enzymatic hydrolysis of cotton fibers: modelling using empirical equation. *J Cot Sci* 8:254–260
- Sasikumar E, Viruthagiri T (2010) Simultaneous saccharification and fermentation (SSF) of sugarcane bagasse—kinetics and modeling. *Int J Chem Biol Eng* 3(2):57–64
- Sousa R Jr, Carvalho ML, Giordano RLC, Giordano RC (2011) Recent trends in the modeling of cellulose hydrolysis. *Braz J Chem Eng* 28(04):545–564
- Teeri TT (1997) Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. *Trends Biotechnol* 15:160–166
- Van Dyk JS, Pletschke BI (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes—factors affecting enzymes, conversion and synergy. *Biotechnol Adv* 30(6):1458–1480
- Van Dyk JS, Gama R, Morrison D, Swart S, Pletschke BI (2013) Food processing waste: problems, current management and prospects for utilisation of lignocellulose component through enzyme synergistic degradation. *Renew Sust Energ Rev* 26:521–531
- Vasquez MP, Da Silva JNC, De Souza Jr MB, Pereira N Jr (2007) Enzymatic hydrolysis optimisation to ethanol production by simultaneous saccharification and fermentation. *Appl Biochem Biotechnol* 136–140:141–154
- Voragen A, Coenen G-J, Verhoef R, Schols H (2009) Pectin, a versatile polysaccharide present in plant cell walls. *Struct Chem* 20:263–275. doi:[10.1007/s11224-009-9442-z](https://doi.org/10.1007/s11224-009-9442-z)
- Wang Z, Xu J, Cheng JJ (2011) Modelling biochemical conversion of lignocellulosic materials for sugar production: a review. *BioRes* 6(4):5282–5306
- Zhang S, Wolfgang DE, Wilson DB (1999) Substrate heterogeneity causes the nonlinear kinetics of insoluble cellulose hydrolysis. *Biotechnol Bioeng* 66(1):35–41
- Zhang X, Tu M, Paice M, Sacciadis G, Jiang Z, Jemaa N, Thibault A (2010) Bioconversion of knot rejects from a sulphite pulp mill to ethanol. *BioRes* 5(1):23–42