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Bio-production of Lactic Acid from Palm Kernel Cake and Kinetic Prediction through Artificial Neural Networks: A Preliminary Study

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Abstract: In this study, palm kernel cake (PKC), a byproduct of palm oil milling, was enzymatically hydrolyzed and used as a raw material in lactic acid (LA) production. The commercial enzyme Cellic® CTec2 was used to release reducing sugars for subsequent LA fermentation by *Actinobacillus succinogenes*. Enzymatic hydrolysis resulted in a decrease in crystallinity of the PKC slurry from 31.9% to 16.2%. The fermentability of the resulting PKC hydrolysate was further evaluated in two stages of the aerobic-anaerobic phase. It was found that the productivity achieved after 52 h of fermentation was 0.75 g/L.h. Moreover, the artificial neural networks (ANN) model was used to predict LA productivity, wherein the coefficient correlation (R^2) value was 0.9976 and the best root mean square error (RMSE) value was determined in 3 layers and 15 neurons. This work lends evidence to the use of ANN as a plausible model for the prediction of LA production rate based on a biorefining perspective.

Keywords: *Actinobacillus succinogenes; Lactic acid; palm kernel cake; Fermentation; Artificial neural network (ANN)*

1. Introduction

Oil palm plantations generate a huge amount of biomass in Malaysia. Currently, the total production of fresh fruit bunch (FFB) and crude palm oil (CPO) was 74.5 and 15.4 million tons, respectively. The production of palm kernel oil was 3.9 million tons during the corresponding period (Hashim et al. 2012). The locally-harvested biomass is available throughout the year and could potentially be used in the production of high-value hydroxycarboxylic acid (Tan et al. 2018).

The production of lactic acid (LA) from bio-renewable sources is a possible solution to improve market competitiveness. LA, being one of the most important hydroxycarboxylic acids, has numerous applications in the food, bioplastic, pharmaceuticals, cosmetic and chemical industries (Krishna et al. 2018). It is estimated that 150,000 tons per year of LA were produced worldwide, with 90% of which was derived from the fermentation involving LA

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bacteria (LAB) such as *Lactobacillus, Streptococcus, Pediococcus, Carnobacterium* and *Enterococcus.* However, there is a lack of evidence that *Actinobacillus succinogenes* could also be a potential LA producer (Li et al. 2010).

Actinobacillus succinogenes is a Gram-negative, facultatively anaerobic, and mesophilic which is isolated from the bovine rumen (Luthfi et al. 2017). *A. succinogenes* has many advantages, including the ability to use a diverse range of carbon sources including glucose, xylose, fructose, sucrose, lactose, maltose, and mannose, which can be found in many renewable and inexpensive lignocellulosic feedstocks that contain mixed sugars (Bukhari et al. 2019).

Over the past decade, the artificial intelligence (AI) method has been a powerful tool in the fourth industrial revolution era. Several AI modelling techniques have been used including artificial neural networks (ANN). ANN make up one of two classical modelling techniques based on the experimental data collated, with polynomial regression models representing the other. Although polynomial regression models can approximate the input-output relationship reasonably well for steady-state engine operation, it is not capable of satisfactorily capturing the non-linearity in a global engine model. This is generally because polynomial models can be susceptible to measurement errors like noise and outliers (Turkson et al. 2016). Hence, this study aims to construct the bio-kinetics of LA conversion by *A. succinogenes* through ANN prediction.

2. Materials and Methods

2.1. Determination of enzyme activity

A total of 0.5 mL of commercial enzyme Cellic® CTec2 (Novozymes A/S, Denmark) was added with 1.0 mL of 0.05 M citrate buffer. The solution was added with pieces of filter paper and heated in a water bath for 1 hour at 50˚C. Then, 3.0 mL dinitrosalicylic acid was added and continued heating for 5 minutes. After heating, the sample was put in an ice bath for 10 minutes and 20 mL of distilled water to dilute the solution. 3,5–dinitrosalicylic acid (DNS) reagents were used to identify the simple sugars liberated by identifying the aldehyde group in the final sugar chain (Chu et al. 2012).

Glucose standard curves were plotted using enzyme concentration data obtained with optical density (OD) readings and thereafter the concentration of the reducing sugars was determined following the established method by Kabel et al. (2006).

Enzyme activity was analyzed according to standard procedure as described by Ghose (1987). The values of absorption of samples (after reduction of blank enzyme) into glucose (in mg of glucose produced during the reaction) were recorded.

2.2. Hydrolysis of palm kernel cake

Palm kernel extracts obtained from Kernel Crushing Plant Carey Island, Sime Darby, Selangor, Malaysia, were dried in an oven at 60°C for 18 h to reduce the moisture content (Yan et al. 2009). Cellic® CTec2 enzyme was loaded at 10 FPU/g up to 80 FPU/g. The quantity of enzymes required for hydrolysis of PKC was determined (Mohammad 2019).

Hydrolysis was performed in an incubator shaker set at 50˚C, and 150 rpm and the pH was maintained at 4.8 using 0.05 M citrate buffer (Luthfi et al. 2016). For 3% glucan loading, the enzyme was loaded at 15 FPU/g dried PKC. Hydrolysis samples were taken at specific intervals during hydrolysis (0 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h), and thereafter heated for 15 minutes at 100˚C to terminate the hydrolysis reaction (Luthfi et al. 2016).

2.3. Fermentation by *Actinobacillus succinogenes*

A. succinogenes purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) was inoculated in the growth medium of brain heart infusion (BHI) broth (Luthfi et al. 2016). For LA fermentation, the media used were (in g/L): yeast extract (30), urea (2), magnesium chloride (2), calcium chloride (1.5), sodium phosphate (4.4), and sodium phosphate (3.3). Inoculum of 5% (v/v) was added in a sterile condition.

A. succinogenes was cultivated in an incubator shaker in a 500 mL flask containing 300 mL fermentation medium at 37°C. The PKC hydrolysate was diluted to obtain 21.4 ± 2.9, 30.7 ± 1.1, 43.2 ± 2.7, and 51.1 ± 0.3 g/L of sugars as the main carbon source. In the first phase of aerobic fermentation, the pH of the media was maintained at 6.7-7.0 with the supplementation of 10 M NaOH solution and the agitation was set at 200 rpm for 14 h. In the second stage of anaerobic fermentation, a total of 50 g/L of $MgCO_3$ was supplemented to the media to stabilize the pH and the operation was set at 40 rpm (Li et al. 2010). The extent of LA production was determined periodically through high-performance liquid chromatography (HPLC) analysis.

2.4. Input and target values for ANN

The input neurons for the study were determined from the experimental parameter. The output neuron was the productivity of LA as outlined in Table 1.

Table 1. Classification of Input and Target Values

Input Neuron		Target Values
Concentration of Sugars (g/L)	Concentration of LA (g/L)	Productivity (g/L.h)
21.4	11.85	1.30
30.7	11.15	1.72
43.2	24.93	1.11
51.1	11.36	2.78

The neural network framework for evaluating the productivity of LA was developed using the MATLAB software version R2008a. The concentration of sugar consumed and the productivity of LA were retrieved and referred from the experimental investigations for developing and solving the neural network using MATLAB R2008a.

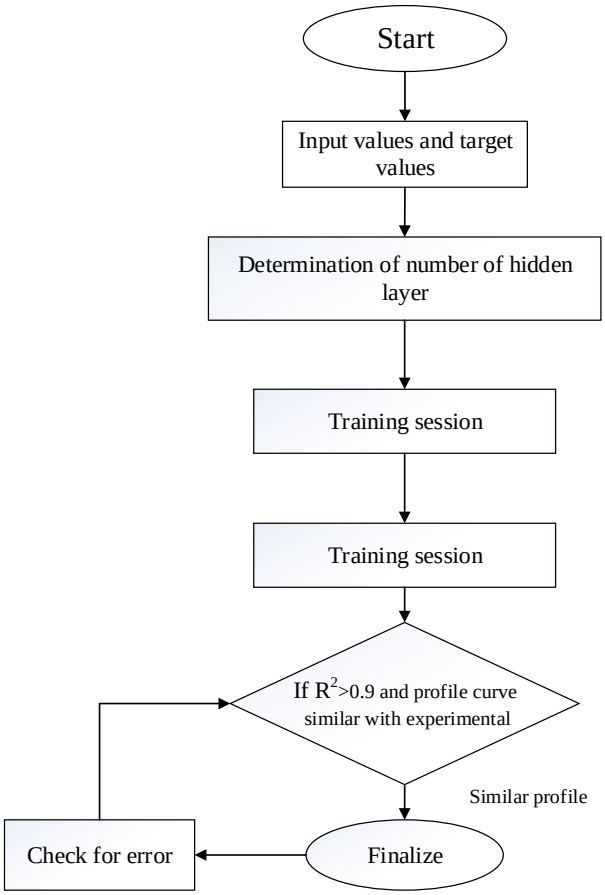

Figure 1. Flowchart using MATLAB Neural Network Toolbox

3. Result and Discussion

3.1. Characterization of palm kernel cake (PKC)

X-ray diffraction (XRD) was used to measure the degeneration of lignocellulose components as shown in Figure 2. Certain classes of hydrolytic enzymes were able to dissolve cellulose (Roslan et al. 2014; Brandt et al. 2013). The biomass is organic solid, and its interaction with organic liquids involves several physical and chemical processes of reaction that include several orders of magnitude in the scale (Zhang et al. 2014).

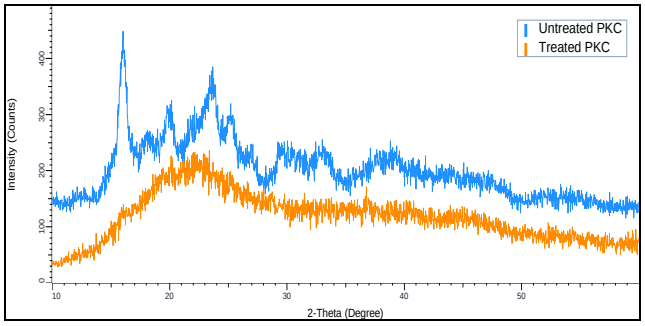

Figure 2. X-ray diffraction patterns for raw untreated and enzymatically treated PKC

As seen in Figure 2, the diffraction peaks of PKC before and after the enzymatic hydrolysis indicated that some decomposition occured in crystalline and amorphous cellulose zones. The degree of degeneration before treatment was 31.9%. After the enzymatic treatment was applied, the diffraction pattern showed a reduction of 16.2% crystallinity. During the pre-treatment of lignocellulose biomass, the amorphous phase of cellulose is fractionated from the original structure of the polymeric carbohydrate matrices (Thulluri et al. 2013). As a result, amorphous substance degrees increased from 68.1% to 83.8% after enzyme treatment. This resulted in the enhancement of biocatalyst accessibility (Binod et al. 2012).

3.2. Fermentation for LA production

The fermentation using *A. succinogenes* 130Z was carried out with different glucose concentrations from the PKC hydrolysate. The flask was shaken at 200 rpm for the first 14 h. Thereafter, the media containing magnesium carbonate was added to convert the phase from aerobic conditions to an anaerobiosis and shaken at 150 rpm for 54 h. Both phases were carried out at 37°C (Li et al. 2010). Figure 3 shows the time-course profile of LA production.

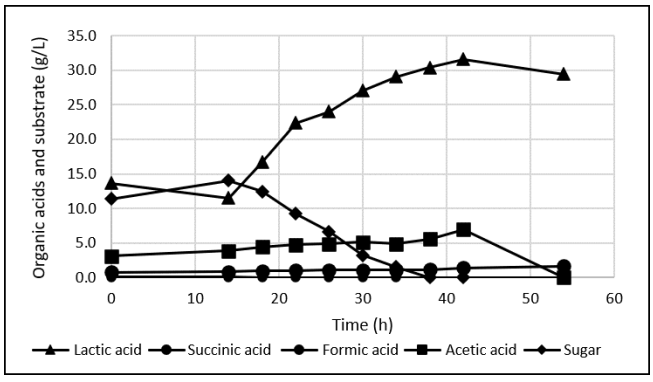

Figure 3. Time-course profile of LA production

As could be seen in Figure 3, it was found that the concentration of LA increased after converting the media from aerobic to anaerobic, which was in agreement with a

previous study (Li et al. 2010). Zou et al. (2011) found that higher levels of dissolved CO_2 solution had a substantial effect on the production of succinic acid. CO_2 transport to cells was kinetically defined via at least four equilibrium equations (Lu et al. 2009).

In the indigenous metabolic pathways, *A. succinogenes* changed flux from C4 to C3 under aerobic conditions. The productivity of LA improved without genetic modification in the subsequent anaerobic cultures. In addition, the specificity of these changes is another benefit of the production of LA, as it produces pure stereoisomers, L-LA. In a two-phase test, the glucose conversion by *A. succinogenes* exceeded 90%. Accordingly, pyruvate is the key source of C3 flux as LA precursor. This can be converted from other substrates as well as from PEP. Pyruvate, for example, can be obtained from malate (McKinlay et al. 2010). Enzyme tests revealed that in two-phase cultivation, the activity of LA dehydrogenase (LDH) was nearly 18 times higher than that obtained in one-phase anaerobic culture. Consequently, higher LDH production in *A. succinogenes* is a significant limiting factor in the rise in C4 flux, and increased CO_2 addition may contribute to the conversion of LA from glucose (Li et al. 2010).

3.3. Productivity of LA

Figure 4 shows LA production with respect to different sugar concentrations. The trend for LA production increased as the concentration of sugar increased. In terms of the composition of fermentation metabolites, LA was higher than the by-product formations. The by-products of fermentation included acetic acid, succinic acid, formic acid and acetic acid. It can be seen that the two phases of fermentation, i.e., aerobic to anaerobic resulting in LA accumulation as the main product (Li et al. 2010). Productivity of LA also increased from 0.37 to 0.75 g/L.h, with the increase in sugar concentrations from 21.4 to 51.1 g/L.

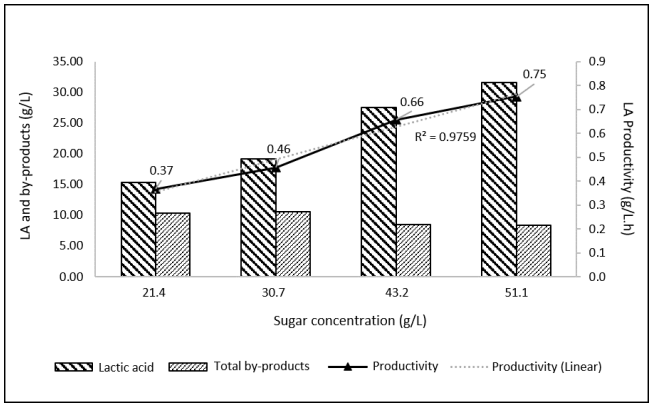

Figure 4. LA production with respect to different sugar concentrations

3.4. Potential of PKC hydrolysate in LA production

The price of PKC consisting of 71.54% of total sugar and glucose were around RM 0.90/kg and RM 1.66/kg, respectively (Shukor et al. 2016; Chai 2013). Assuming a sugar yield of 76% (w/w) from PKC biomass (or glucose), the raw material cost of the process was estimated to be RM 1.11/kg (or RM2.17/kg) of LA. Hence, the cost of PKC as raw material for the production of LA was RM 1.11/kg LA, or 53% of the cost of using glucose.

Raw material availability is critical for the continued release of carbon sources in the production of LA. The use of biomass as a carbon source has many advantages in terms of the sugar produced, the low cost, and the availability of raw materials. The use of PKC as a carbon source would definitely reap the benefits of the oil palm industry (Lim Siew Ling 2001).

3.5. ANN modelling in predicting the productivity of LA

In the experiment, different concentration of sugars were tested. The fermentation took about 54 h to complete the reaction. Table 1 shows the detailed description of the ANN framework used for this study.

Table 2. Detailed description of the ANN framework

Specification	Description
Toolbox	Artificial neural network (ANN)
Parameter tested	Concentration of glucose (g/L)
	Concentration of LA (g/L)
	Productivity
Type of network	Feed-forward back propagation/perceptron
Input layer	Concentration of glucose (g/L)
	Concentration of LA (g/L)
Output layer	Productivity
Targeted range	Productivity : 0.37 – 0.8 (g/L.h)
Neuron	15 layer

Since the reaction time was 54 h, the operation was performed once a week. The productivity was 0.37 g/L.h, 0.46 g/L.h, 0.66 g/L.h, and 0.75 g/L.h for a concentration of 21.4, 30.7, 43.2, and 51.1 g/L, respectively. The amount of glucose directly affected the productivity of LA as the bacteria grew and consumed the substrate until the LA production was halted.

Table 3 shows an increase in the correlation coefficient (R^2) which coincides with the study of Rostampour et al. (2013). This corroborated that the performance model increased as the number of hidden neurons increased. The number of correlation coefficients must be over 0.9 for validation, and hence, the first reading was omitted (Gandhi et al. 2016).

Table 3. Regression number with different numbers of layers and neuron

Number of layers	Number of neurons	R^2
1	10	0.76589
2	10	0.95864
3	10	0.95960
1	15	0.98310
2	15	0.98807
3	15	0.99760
4	10	-0.69350

Based on the ANN with 3 hidden layers and 15 neurons, the correlation coefficient was 0.99760. The number of layers affected the value of R^2. Excessive numbers of neurons in the hidden layer can cause problems that are too straightforward, resulting in good network learning and data memorization, but lack the ability to generalize (Rostampour et al. 2013). Therefore, the number of neurons selected were only 10 and 15.

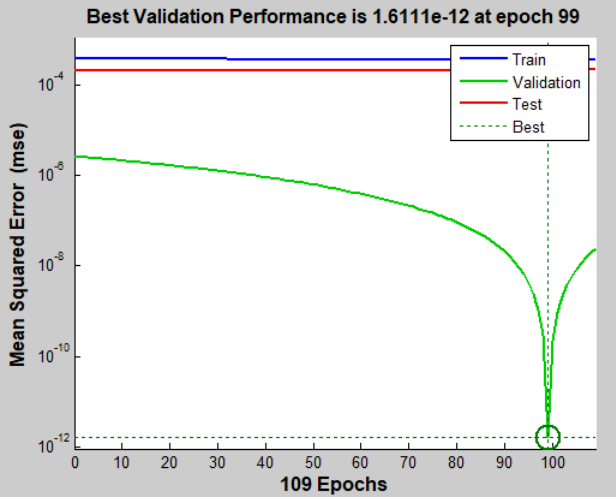

Figure 5. The best performance for number of layers = 3 and number of neurons = 15

The number of nodes in the input and output layers corresponds to the number of inputs and variable outputs in the data set. The ideal number for the hidden layer must go through several experimental methods (Esmaeelnejad et al. 2015). The one with 4 hidden layers showed the negative value of the correlation coefficient, indicating it is not suitable for use in this experiment. Hence, the best performance was attained for 3 number of layers and 15 number of neurons with the regression line at 0.9976.

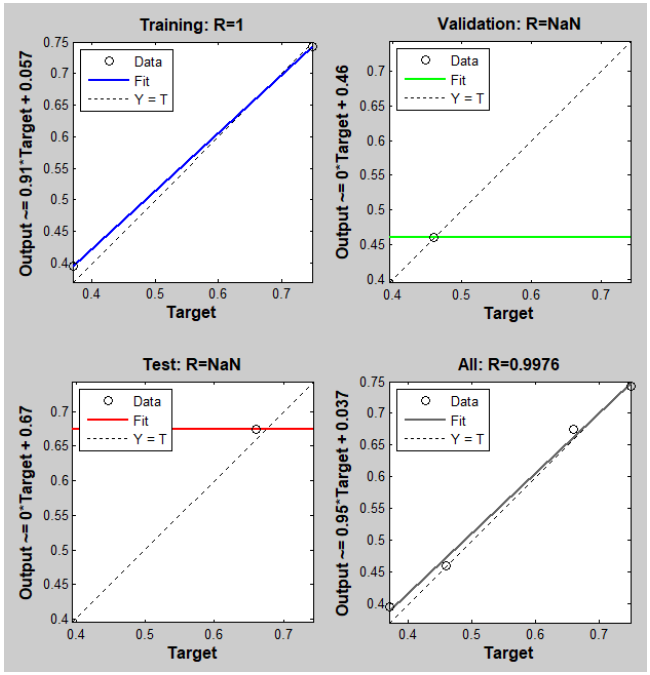

Figure 6. The regression line

4. Conclusion

This study was attempted to observe the extent of hydrolysis of sugar in oil palm residues. The degree of deterioration after treatment was reduced to 16.2% from 31.9%. Amorphous cellulose degrees showed an increase from 68.1% to 83.8% after enzyme treatment. The reduction of amorphous substance had remarkably improved the enzymatic efficiency. The highest production of LA was attained from 50 g/L glucose concentration. The LA productivity was improved from 0.37 to 0.75 g/L.h with a sugar concentration of 21.4 to 51.1 g/L. Moreover, the ANN modelling showed the best regression line at 0.9976.

5. Acknowledgements

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