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pH prediction for a semi-batch cream cheese fermentation using a grey-box model

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Abstract: Cream cheese, a popular condiment, is widely used in people's daily diet and in dessert making. To ensure high-quality cream cheese production, the pH value is generally used as the indicator to determine the end point of cream cheese fermentation. The inoculation time and time-dependent concentrations of biomass, lactose, lactic acid are all crucial for pH prediction. However, the inoculation time could vary for industrial applications with multiple fermenters. Moreover, the inoculation time impact on fermentation has not been investigated. This paper aims to build a cream cheese fermentation model predicting pH. The model includes a semi-batch kinetic model and an artificial neural network (ANN) model. The outcome of the model will help the cream cheese industries understand the inoculation time impact on fermentation time and organise better fermenter scheduling.

Keywords: artificial neural network; cream cheese fermentation; kinetic model; pH prediction.

1 Introduction

Cream cheese, a popular condiment, is widely used in food preparation, such as sandwiches, salads and making various baked goods and desserts. The consumption of cream cheese has increased sharply in recent years. This increasing trend is due to multiple reasons [1]. Firstly, the urban population and their income have increased, and the attraction of customers to cream cheese has also risen. Another reason is that with the continuous expansion of the fast-food market, the demand for cheese is also increasing. According to a report conducted by [2]; the total market value of cheese worldwide was US $69.7 billion in 2019. It is projected to grow steadily to US $112.8 billion by 2025, with a compound annual growth rate of 8.4% from 2020 to 2025. As a critical part of the cheese market, the global cream cheese market is expected to reach about US $8.3 billion by 2026.

To build a fermentation model of cream cheese, it is important to understand the cream cheese production process. At the beginning of cream cheese production, the milk and cream are mixed together and pasteurised. After pasteurisation, the mixture is added to an empty vessel. This filling process can last about 2 h in a fermentation plan. At some point during the filling time, an ice cube containing starter culture, lactic acid bacteria, is added to the feeding stream. With the melt of the ice cube, the culture is released into the mixture gradually, and the fermentation starts. When the fermentation ends, the mixture goes through curd cooking and de-gas. Then, the cheese whey, as the by-product of cream cheese, is separated. After adding gum and salt to the mixture, the cream cheese is ready for packaging.

During the fermentation process, the lactic acid bacteria consume lactose and produce lactic acid, and the pH value decreases with the increase of lactic acid concentration. As a result, pH value will show the progress of cream cheese fermentation. Traditionally, most cheese industries use the pH value to determine the end time of fermentation. A too low pH value during fermentation may cause the growth of mold after packaging. A too high pH value will increase the risk of pathogen formation for consumption [3]. In terms of flavour, if the termination

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pH is too high, the flavour of cream cheese will be deficient. In contrast, low termination pH will cause an overly acidic flavour [4]. The pH prediction becomes a key to improve fermentation quality, scheduling and plant throughput. In this paper, a grey box model including a fermentation kinetic model and ANN model is developed to predict the fermentation pH values.

There are three popular models: white-box models (WBM), black-box models (BBM), and grey-box models (GBM). Akin et al. [5] proposed a static WBM to determing the pH value during alcoholic fermentation. This model requires the concentration of sugars, main organic acids, mineral elements, nitrogen compounds, ethanol and temperature. A BBM is based on data from the experiment, which is also known as an empirical model. BBMs have been applied to measure the physical properties of cheese, food quality and predict moisture in the cheese [6–9]. Latrille et al. [10] built a feedforward ANN model (BBM type) to provide a reference fermentation pH curve in lactic acid batch fermentations. Ebrahimpour et al. [11] proposed a feedback ANN model to predict pH values for cream cheese fermentation. The model could use the previous pH data or previous outputs of this BBM to predict the pH change in future time steps. To obtain reliable BBM model suitable for different fermentation conditions (e.g. The initial concentration of biomass, lactose or lactic acid), large amount data are required, it limits the BBM applications. Since the GBM doesn't request a lot of data comparing to the BBM, Li et al. [12] developed a GBM to predict the cream cheese pH. However, the model did not consider the impact of inoculation time. Differing inoculation times result in different volumes of milk in the fermenter at the beginning of fermentation. Therefore, the initial biomass concentration will differ.

This paper has built a grey box model to predict the pH change during fermentation. This involves inputting biomass concentration, lactose concentration, lactic acid concentration, and inoculation starting volume. The grey box model includes two models: (i) a semi-batch kinetic model to predict the biomass, lactose, and lactic acid concentration during both feeding and fermentation stages; and (ii) Since the LSTM has been proven to predict pH changes effectively [13]. The LSTM is a special kind of recurrent neural network (RNN). It could solve complex, long-time-lag tasks [14]. Therefore, it is widely used to predict data based on time series data. We use the LSTM model trained by the outputs from the kinetic model to predict the pH change with different initial biomass, lactose, lactic acid concentration and inoculation time.

2 Materials and methods

2.1 Cream cheese fermentation

1.5 L Standard Blue Top Anchor Milk from Fonterra Co-operative Group Limited and 0.5 L Countdown Fresh Cream from Woolworths Limited were mixed well and preheated to 30 °C. Then the initial volume mixture was added to the fermenter. The rate of the stirring pump was set to the maximum 120 rpm so as to stir as evenly as possible. Two pumps were used to add the remaining mixture of milk into the fermenter at a flow rate of 1 L/h. At the same time, the starter culture was also added to the fermenter. The cheese culture (Stock keeping unit: 73,712-RRP) was bought from the local store, Mad Millie. Each sachet contained freeze-dried *Lactococcus lactis*. The activity was about 2.4 U. The first sample was taken 5 min after adding starter culture into the fermenter. Two 10 mL samples were taken every 2.5 h. One sample was for the measurement of lactose and lactic acid concentration. Another sample was for the measurement of biomass concentration.

2.2 Measuring biomass, lactose, lactic acid concentration

There are two strains of bacteria in the starter culture, *L. lactis* subsp. *cremoris and L. lactis* subsp. *lactis.*, which were all *L. lactis.* For them to grow, the optimal temperature was approximately 30 °C and pH 6.3–6.9 [15]. There are two popular methods to measure the bacteria concentration, pour plate and spread plate. The pour plate method provides a standard method to calculate the biomass concentration and generate the growth curve of mixed bacteria, while the spread plate method is popular for separating mixed bacteria [16]. As there were two strains in the starter culture, the pour plate method was used to measure the biomass concentration in this experiment. The best statistically significant range for each plate is from 30 to 300 colony forming units (CFU). Therefore, all biomass samples were diluted to 10^{-6}, 10^{-7}, and 10^{-8} in accordance with the preliminary experiments. In order to reduce the dilution error, two plates were poured for each dilute. Then, 15 mL of MRS agar was added into the petri dish with gentle shaking to spread bacteria evenly. All colonies were countable after 48 h of inoculation. The calculation of biomass concentration was defined by Equation (1):

$$\text{biomass concentration} = \frac{\text{CFU} \times \text{dilution factor}}{\text{aliquot}} \ [\text{CFU/mL}] \tag{1}$$

The lactic acid concentration was measured by the assay kit provided by Megazyme [17]. Megazyme's lactose assay kit is used to determine the lactose concentration [18].

2.3 Kinetic modelling and LSTM training

After the fermentation experiment, the volume (V_e), pH, and the concentrations of biomass (X_e), lactose (S_e), lactic acid (P_e) were collected. This data was then used to build kinetic models. The kinetic model with the best performance was chosen to train the long short term memory (LSTM) network. Through inputting the inoculation starting volume (V_0), and the initial concentration of biomass (X_0), lactose (S_0), lactic acid (P_0), the kinetic model could generate a corresponding concentration (X_t, S_t, P_t) which had the same time series with a pH value. Experiment data were divided into two sets, training set (*tr*) and test set (*te*). After that, the LSTM network was trained and evaluated by the two sets, respectively. The proposed model structure is shown in Figure 1.

2.3.1 Kinetic modelling: The measured concentrations of biomass, lactose and lactic acid were used to verify the accuracy of three kinetic models, where the model outputs are the integrated trends as simulated by the Matlab rapid proto-typing environment. The kinetic model with the best performance was used to train the LSTM network.

The batch fermentation kinetic model consists of the change of biomass, lactic acid, lactose mass and volume changing rate. The increase of biomass mass was from three parts. One part is the biomass from the mixture feeding stream. The second part was from externally added biomass. The remaining increasing mass was from the self-reproduction of bacteria. The increase of product mass was from the lactic acid in the mixture feeding stream and biomass production. The mass change of lactose came from a dual effect: it was increased by the feeding stream of the mixture while being continuously consumed by bacteria. In previous work, the modified Boonmee's model [19] was proven to have the best performance [20]. The kinetic model can be expressed as,

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu_{\max} X\left(1 - \frac{P - P_{ix}}{P_{mx} - P_{ix}}\right) + F\frac{X}{V} + X_{\text{mixture}} \tag{2}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \alpha\frac{\mathrm{d}X}{\mathrm{d}t} + q_{p\ \max} X\frac{S}{VK_{.\text{sp}} + S} + F\frac{P}{V} - \alpha\frac{X}{V} + P_{\text{mixture}} \tag{3}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -q_{s\ \max} X\frac{VK_{is}}{VK_{is} + S} + F\frac{S}{V} + S_{\text{mixture}} \tag{4}$$

$$\frac{\mathrm{d}V}{\mathrm{d}t} = F\,(V < 2L), \text{else}\ F = 0 \tag{5}$$

where X is the mass of biomass, P is the mass of lactic acid, and S is the mass of lactose, V is volume, F is the flow-in rate, $\mu_{\max}$ is the maximum growth rate, α is the inhibition constant, $X_{\text{mixture}}, P_{\text{mixture}}, S_{\text{mixture}}$ are the concentration of biomass, lactic acid and lactose in the mixture. These equations are modified to include the effects of lactose limitation and inhibition, as well as lactate inhibition. Lactose limitation constant (K_{is}) follows the Monod model, while lactose inhibition follows a typical non-competitive inhibition model. Lactic acid inhibition is considered to occur in a linear manner, with an initial value (P_{ix}) being a threshold lactate concentration before any inhibition occurs and a value P_{mx} being the maximum inhibitory value. $q_{p\ \max}$ is the maximum specific lactic acid production rate. $q_{s\ \max}$ is the maximum specific lactose utilisation rate. When the current volume is less than 2 L, the F is 1 L/h. Moreover, the feeding stream stops after the volume reaches 2 L, which is the same for $X_{\text{mixture}}, P_{\text{mixture}}, S_{\text{mixture}}$.

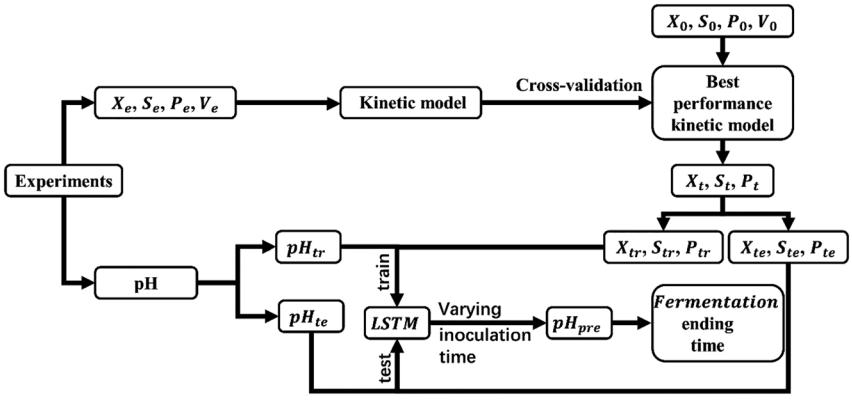

Figure 1: The proposed grey box model structure.

2.4 LSTM training

In the LSTM network, the information could flow along with the cell state without change. The forget gate and input gate could filter the information. As a result, LSTM could add or delete information to the cell state. At time t, the output h_{t-1} from previous cell and the information X_t flows into the cell. The forget gate can decide whether to delete some parts of the information. Then the filtered information was transferred through the input gate and added to the cell state. The output gate will decide which part of the information will be output. In Figure 4, the output is h_t. The cell state at time t will be transferred to the next cell to predict the cell state at time $t + 1$. General LSTM introduction is provided by Saxena [21].

The LSTM network structure of pH prediction during cream cheese fermentation is shown in Figure 2.

where X_0, P_0, S_0 is the initial concentration of biomass, lactic acid and lactose, V_0 is the initial starting volume, pH (t) is the change of pH value by time.

In order to build the LSTM network, five experiment datasets were used in the network training. The number of hidden neurons was set to be 20. The solver chosen has the initial learning rate of 0.01 and the gradient threshold of 1.

The LSTM training process is shown below:

- Step 1: Input the initial conditions of all 5 set of data into the best performance kinetic model. Then the kinetic model will give feedback for the prediction of concentration change for each set of data.
- Step 2: Input the pH and time value during the fermentation process.
- Step 3: Find the value of the predicted concentration at the corresponding time.
- Step 4: Separate all the data into two groups. 80% of the data is a training group. The left 20% is the test group.
- Step 5: Train the LSTM net with the training group and test by the test group.

To evaluate the performance of the LSTM network, the remaining 20% of Dataset 1 was used as the test data. The SSE value was calculated to evaluate the performance of the model.

$$\mathrm{SSE} = \left(\Sigma i \left(X_t - X_p\right)^2\right) / n \times 100 \tag{6}$$

where i is the different data point, X_t is the test data, X_p is the prediction value, n is the total sample size. The training process and prediction result is shown Figure 3.

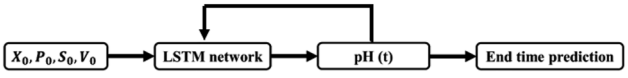

Figure 2: The LSTM network to predict pH change.

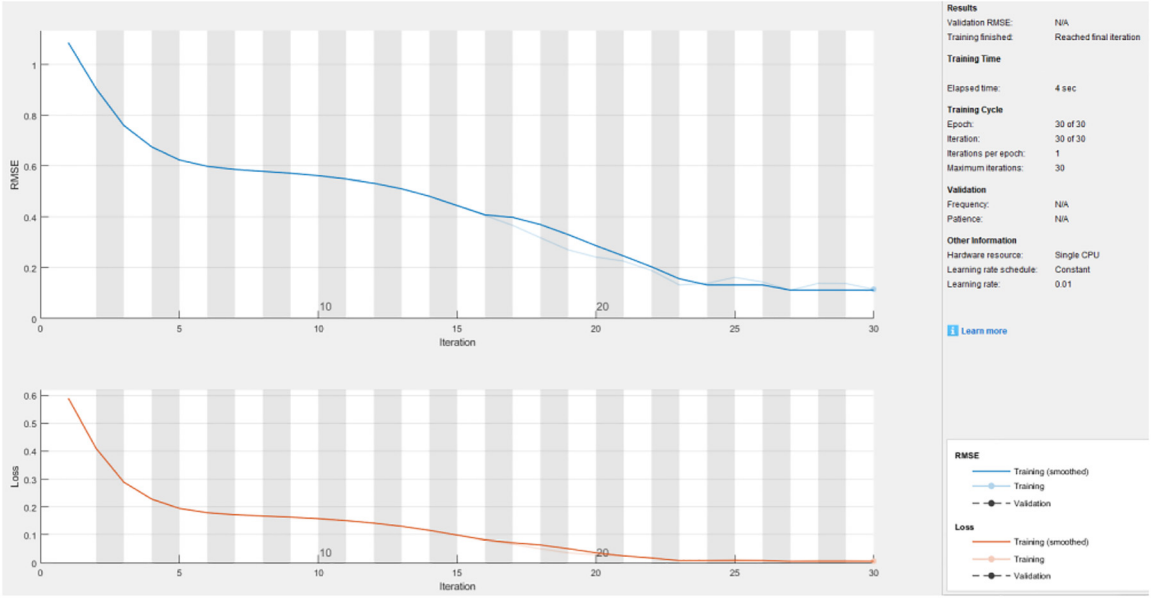

Figure 3: The training process of LSTM.

3 Results and discussion

3.1 Initial condition and fermentation data

Initial conditions of the fermentation experiments are summarised in Table 1.

The final results for lactose concentration (S), biomass concentration (X) and lactic acid concentration (P) are shown in Figure 4.

The fermentation ending indication is pH = 4.6. The change of pH for all five sets of experiments is shown in Figure 5.

3.1.1 Fermentation experimental data discussion

At the beginning of the experiment, the biomass was added to the fermenter at the inoculation starting volume. The remaining milk mixture continued to be added to the fermenter. In Data set 1 (initial 1 biomass 05 L) and set 4 (HL 1 biomass 05 L), the inoculation starting volume was 0.5 L. The biomass concentration decreased at the beginning as the milk mixture was continuously added, and the biomass concentration was diluted. When the growth rate of lactic acid bacteria was fast enough to offset the effect of adding milk mixture, the biomass concentration started to grow. In Data set 2 (initial 1 biomass 15 L), set 3 (initial 2 biomass 15 L) and set 5 (HL 1

Table 1: The initial conditions of the five datasets.

	Initial 1 biomass 0.5 L	Initial 1 biomass 1.5 L	Initial 2 biomass 1.5 L	HL 1 biomass 0.5 L	HL 1 biomass 1.5 L
Biomass (g/L)	0.137	0.040	0.082	0.102	0.052
Lactose (g/L)	41.246	44.102	45.563	46.285	44.775
Lactic acid (g/L)	0.024	0.023	0.021	0.032	0.032
Inoculation starting volume (L)	0.5	1.5	1.5	0.5	1.5

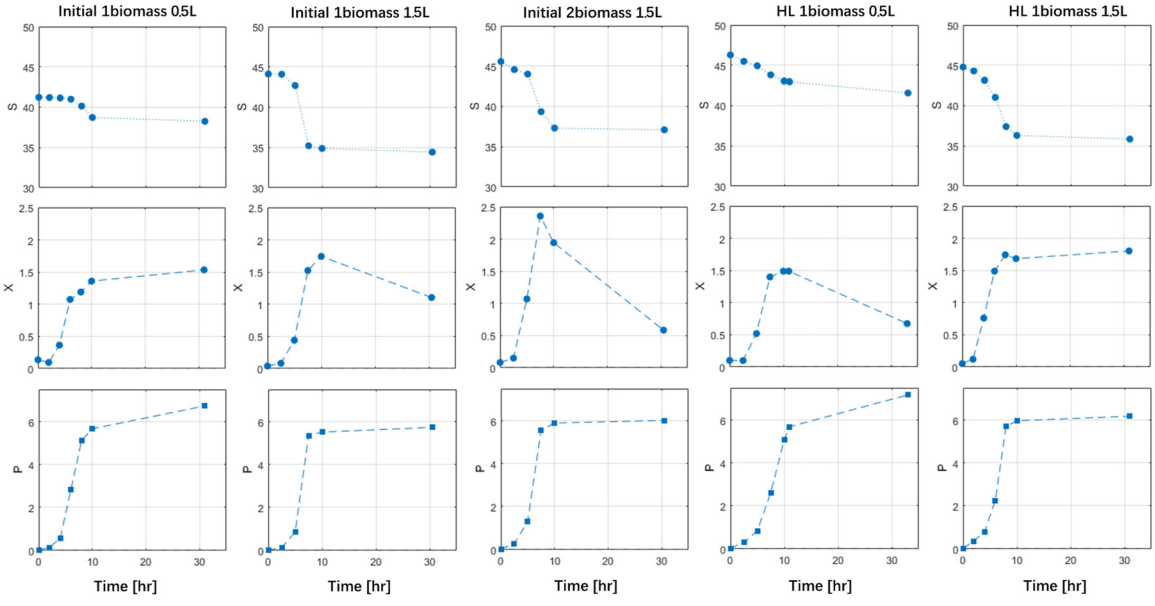

Figure 4: Experimental data.

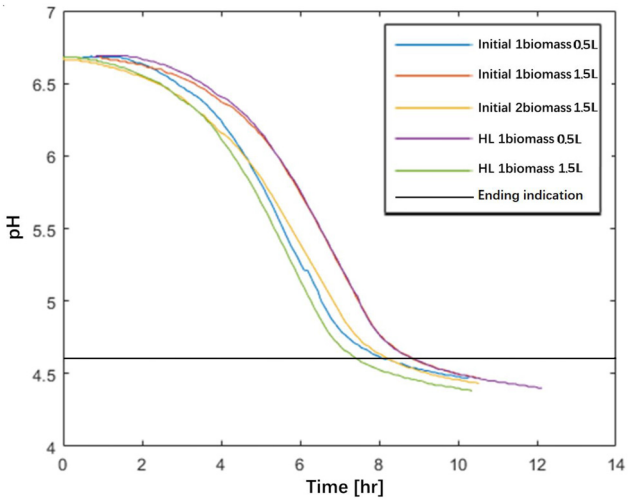

Figure 5: The change of pH for all five experiment sets.

biomass 15 L), the inoculation starting volume was 1.5 L. The biomass concentration increased between the first two data points. This was different from the experiment that started with 0.5 L biomass because the addition of milk mixture stopped between these two data points, and the growth rate was fast enough to offset the dilution of the added milk mixture. Consequently, the biomass kept increasing and reached the maximum concentration point. This maximum concentration of biomass mainly depended on pH value.

With the increase of biomass concentration, the pH change rate also increased. However, a low pH value also inhibited the growth of biomass. In this case, the pH value kept steady at the beginning due to the biomass concentration being low. Also, the production rate of lactic acid was slow. With the increased concentration of biomass, the pH change rate became faster. When the pH rate reached about 4.8, the pH inhibition of biomass appeared. The biomass stopped growing and then died after pH reached 4.8. With the decreased concentration of biomass, the lactic acid production rate decreased, and as a result, the pH kept steady at around 4.3.

Compared to Data set 1 (initial 1 biomass 05 L), set 4 (HL 1 biomass 05 L) took the longest time to reach pH 4.6. This could be caused by the inhibitory effect of high lactose concentration on bacterial growth. This result is also supported by a similar experiment carried out by Fu & Mathews [22]. The cell growth of *Lactobacillus plantarum* was inhibited under high lactose concentration and low pH condition. *L. plantarum* and the cheese starter culture, *L. lactis,* are all lactic acid bacteria and belong to *Lactobacillales*. Consequently, the cheese starter culture could also be inhibited by high lactose concentration. Data set 5 (HL 1 biomass 15 L) did not show the high lactose inhibition because its initial lactose concentration, 44.78 g/L, was lower than that of set 4 (HL 1 biomass 05 L), 46.29 g/L and the mass of initial biomass in data set 5, 0.078 g, was higher than that of set 4, 0.051 g.

3.2 Kinetic modelling

The prediction and cross-validation results are shown in Figure 6 and Table 2.

The average validation SSE: SSE = 34.96.

The Akaike information criterion: AIC = 69.31.

3.2.1 Kinetic modelling discussion

The modified Boonmee et al.'s model predicted the pH change with the least SSE, 34.96, and the least AIC, 69.31. It was able to capture the changing trend of lactic acid concentration in the beginning 12 h. As shown in Figure 5, the decreasing trend of pH gradually eased around 4.5. Due to the pH inhibition, the biomass concentration should stop increasing at that time. Consequently, the biomass concentration should show an 'S' curve, growing period, steady period and death period. The biomass concentration growth was slow at the beginning of the experiment

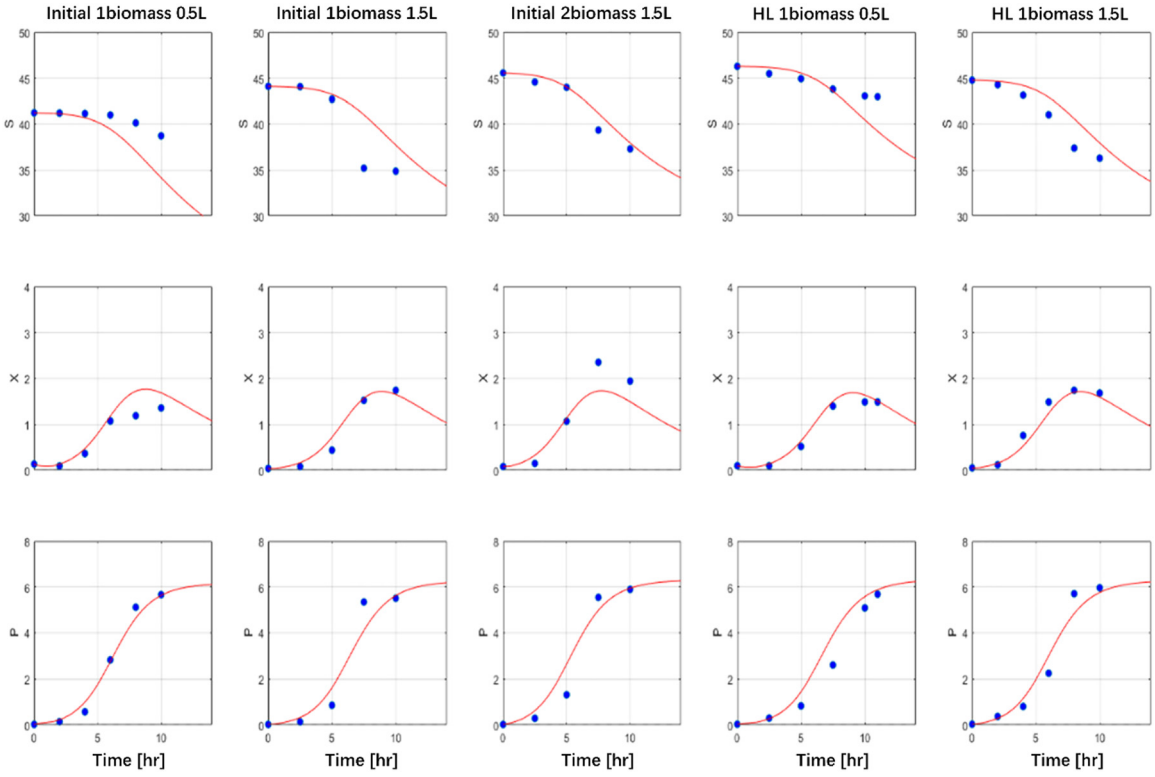

Figure 6: The prediction results of kinetic model 1.

Table 2: Optimised parameters for the kinetic model 1.

Parameter	Value	SD
μ_{max}	1.54×10^{-10}	0.0001
P_{ix}	4.80	0.0013
P_{mx}	4.80	0.0325
α	1.33	0.0001
$q_{p\ max}$	3.75×10^{-5}	0.0001
K_{sp}	−27.50	3.3258
$q_{s\ max}$	2.10×10^{-4}	0.0001
K_{is}	5.41×10^{5}	218

due to the adding of the milk mixture. After reaching the maximum biomass concentration, the lactic bacteria began to enter a period of death. As a result, the biomass concentration decreased. The experiment data also proved this trend. The biomass curve of the modified Boonmee et al.'s model also followed the standard biomass curve, rising, levelling off and then declining at the end. Furthermore, the predictions of lactic acid and lactose concentration were also in line with the above-mentioned principles. When the biomass concentration increased, lactose consumption and the growth of lactic acid concentration also become faster. When the biomass concentration decreased, lactose consumption and the growth of lactic acid concentration correspondingly became slower.

3.3 LSTM training

3.3.1 LSTM training result

Test 1: To evaluate the performance of the LSTM, the remaining 20% of data set 1 was used as the test data.

Test 2: The whole group set 2 data was also inputted into the net to evaluate the performance.

Test 3: Another separate experiment with a similar initial condition to set1 was also done to evaluate the performance of the LSTM network. The initial conditions of the experiment are shown in Table 3. The LSTM prediction results of Test 1, 2 and 3 are shown in Figure 7. The redline is prediction from model. The blue line is the test data. The black line is the fermentation ending indication.

In Test 1, the LSTM network predicted the pH trend, as well as the difference between the predicted value and true value. The average SSE value for the prediction was 0.32. The prediction of fermentation time when the pH reached 4.6 was 8.381 h. The real finishing time was 8.235 h. The difference between them was 1.7%. The R^2 value was 0.99.

In Test 2, the LSTM network predicted the pH trend, as well as the difference between the predicted value and true value. The SSE value of the prediction was 0.33. The prediction of fermentation time when the pH reached 4.6 was 9.272 h. The real finishing time was 8.862 h. The difference between them was 4.4%. The R^2 value was 0.99.

In Test 3, the LSTM network predicted that the cream cheese fermentation would reach the optimum pH of 4.60 at 8.26 h, and the actual finishing time was 8.75 h. The difference between the predicted finishing time and the actual finishing time was 0.49 h which was a 5.6% difference. The SSE value for the prediction was 0.37. The R^2 value was 0.97.

3.3.2 LSTM training discussion

The training results are summarised in Table 4.

All three tests show that the LSTM could perform the pH change during cream cheese fermentation in a correct trend, dropping slowly at first, then decreasing dramatically, and keeping steady at last. The pH drops

Table 3: The initial conditions of an individual experiment.

Inputs	Test data
Biomass (g/L)	0.13
Lactose (g/L)	41.28
Lactic acid (g/L)	0.022
Inoculation starting volume (L)	0.5

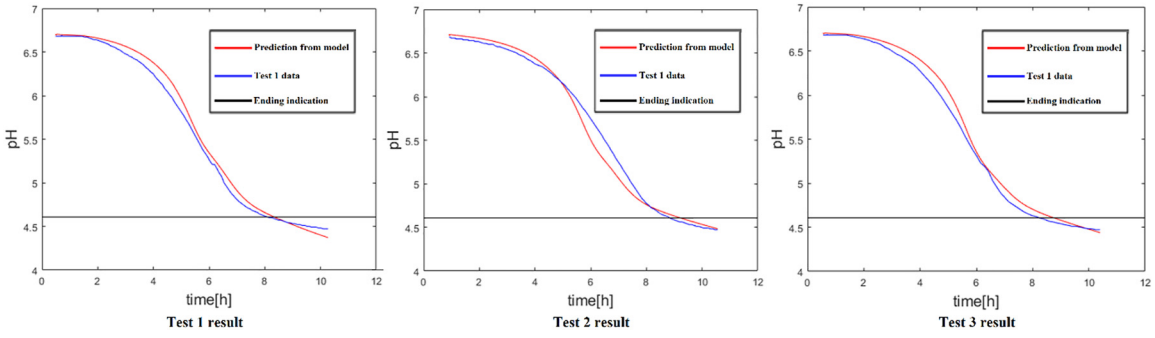

Figure 7: The LSTM prediction results of test 1, 2 and 3.

Table 4: Finishing time from LSTM prediction and actual measurement (unit: hour).

	Prediction	True	SSE	R^2	Difference (%)
Test 1	8.37	8.23	0.32	0.99	1.7
Test 2	9.27	8.86	0.33	0.99	4.4
Test 3	8.26	8.75	0.37	0.97	5.6

slowly at the beginning of the fermentation because the lactic bacteria concentration is low, and the milk-cream mixture is added constantly at that time. After that, the biomass concentration grows exponentially. As a result, there is a sharp increase in the concentration of lactic acid, which led to a dramatic decrease in the pH value. Subsequently, with the decrease of pH, the lactic bacteria are inhibited by low pH (Figure 5). The bacteria concentration stop increasing and begin to decrease. The decline in bacteria concentration causes a decrease in the rate of decline in pH.

In all 3 tests, Test 1 has the best performance with a minimum difference (1.7%) and maximum R^2 (0.99). The prediction end time is 8.37 h and the measurement end time is 8.23 h. This is because Test 1 data set was randomly chosen from Fermentation Experiment: Initial 1 biomass 0.5 L. The remaining data are used to train the LSTM. Therefore, Test 1 has the best prediction result of the fermentation end time. Test 3 has the worst prediction result with a difference (5.6%) and R^2 (0.97). The prediction end time is 8.26 and the real finishing time is 8.75. As Test 3 data is a separate experiment, the LSTM has not been trained by these data. As a result, it has the worst prediction result. With more experimental data training kinetic and LSTM model, the prediction result could be improved.

4 Conclusions

This research has built a grey box model to predict the pH change during fermentation. This involves inputting biomass concentration, lactose concentration, lactic acid concentration, and inoculation starting volume. The grey box model includes two models: (i) a semi-batch kinetic model to accurately predict the biomass, lactose, and lactic acid concentration during both feeding and fermentation stages; and (ii) an Artificial Neural Networks (ANN) model trained by the outputs from the kinetic model to predict the pH change with different initial biomass, lactose, lactic acid concentration and inoculation time.

Thus, this model could enable the cream cheese production plant to better understand the cream cheese fermentation process and the effect of different inoculation times on pH change. Another benefit of this research is that this model could help cream cheese production plants predict the fermentation ending time and provide guidance for the inoculation time.

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