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Advanced Expanded Microbial Kinetics (EMK) Model for Ethanol Production from Mixed Cassava and Fruit Wastes

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Abstract

Bioprocesses are often highly nonlinear due to process variability and complexity of biological systems. In this study, a series of batch experimental study was conducted on ethanol production using combined cassava and rejected mango fruits as a fermentation feedstock. The effects of pH and aeration rate were studied in the experiments. An advanced EMK model was developed to mathematically represent the simultaneous effects of pH and aeration rate on microbial kinetics for a highly nonlinear fermentation. The advantage of this proposed model is that it requires only small number of experimental runs to obtain a high-order order prediction response with an improved accuracy. The results showed that the model can fit the experimental data well within the given ranges of pH and aeration rate used in the study.

Keywords: EMK model; Ethanol production; Cassava; Fruit wastes; pH; Aeration rate.

1. Main text

Over the past decades, the major feedstock for conventional ethanol production has been sugar- and starch-based crops. This heavy reliance on agricultural crops will lead to a long-term competition with human and livestock food consumption \cite{1, 2}. On the other hand, the usage of lignocellulosic materials could relieve the fuel versus food
competition problems. However, the usage of lignocelluloses requires costly pretreatment process to decompose the complex lignin structure which often reduces the productivity [3]. In this research, the usage of fruit wastes (e.g., damaged fruit and peels) has been proposed to address the feedstock problems for long-term sustainability of bioethanol production. The use of fruit wastes represents an advantage over that of lignocellulose because the latter are more readily fermentable and inexpensive. In this study, batch fermentation for ethanol production via *Saccharomyces cerevisiae* (baker’s yeast) in combined hydrolyzed cassava and mango waste was conducted.

The performance of bioprocess is complex, i.e., strong nonlinearity characteristics are frequently caused by process variability and dynamic complexity of biological systems [4]. It is a challenging task to describe the dynamic behavior of the operation of alcoholic fermentation using a simple mathematical model. In this respect, unstructured (macro-scale) models have widely been used for modeling the microbial kinetics in order to describe the microbial growth during ethanol fermentation. The models are mostly empirical which aim to provide the most fundamental observations concerning microbial metabolic processes [5]. For example, the Herbert-Haldane model has often been used to study the behavior of yeast, substrate consumption and ethanol production. This model is simple but can provide a good fitting to the fermentation process data. There are many process parameters that can affect ethanol fermentation, such as pH which has been known to impose a serious impact on microbial activities [6]. Hence, an optimal yeast intracellular pH has to be maintained during a fermentation process [7]. Moreover, in a micro-aerobic or aerobic fermentation, an oxygen supply to culture broth is important as to maintain desired metabolite production and growth of microorganisms [8]. In the present work, the combined effects of both pH and aeration rate on the ethanol micro-aerobic fermentation process has been studied.

The main goal of the present paper is to propose an advanced expanded microbial kinetic (EMK) model to represent the fermentation kinetics in the presence of varying pH and aeration rate. This advanced EMK model can be employed for process simulation, optimization and control purposes.

2. Materials and method

2.1. Experimental setup

A set of experiments was executed in batch mode by using a BIOSTAT A-plus 2 L, MO-Assembly bioreactor. Rejected mango fruits and hydrolyzed cassava were used as a carbon source for the fermentation process. The batch experiments were carried out for a period of about 60 hours until the substrate was used up. The effect of pH and aeration rate (AR) on the rates of microbial growth, substrate consumption and ethanol production were analyzed throughout the experiments. Table 1 shows experimental runs with the values of pH and aeration rate (AR) with a fixed stirrer speed of 240 RPM. Run 0 denoted the baseline experimental conditions. The experimental runs 0 to 4 were used in the advanced EMK model development.

<table>
<thead>
<tr>
<th>Experimental run</th>
<th>pH Value</th>
<th>Coded level</th>
<th>Aeration rate (AR)</th>
<th>Coded level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 0 (baseline)</td>
<td>5.0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Run 1</td>
<td>4.5</td>
<td>-1</td>
<td>0.5</td>
<td>-1</td>
</tr>
<tr>
<td>Run 2</td>
<td>4.5</td>
<td>-1</td>
<td>1.5</td>
<td>+1</td>
</tr>
<tr>
<td>Run 3</td>
<td>5.5</td>
<td>+1</td>
<td>0.5</td>
<td>-1</td>
</tr>
<tr>
<td>Run 4</td>
<td>5.5</td>
<td>+1</td>
<td>1.5</td>
<td>+1</td>
</tr>
</tbody>
</table>

2.2. Medium preparation

1.5 L of medium culture was prepared. First, 150 g of cassava powder was mixed with 562.5 mL of 1 N H₂SO₄ solution. The cassava medium culture was cooked at 121°C for 20 minutes in order to break down the cassava starch into fermentable sugars and then cooled down to room temperature, approximately at 30°C. 1.5 g yeast extract, 3.75
g NH₄Cl, 4.365 g Na₂HPO₄, 4.5 g KH₂PO₄, 0.375 g MgSO₄, 0.12 g CaCl₂, 6.45 g citric acid and 4.5 g sodium citrate were dissolved in 562.5 mL of distilled water and mixed with the hydrolyzed cassava medium culture. 375 mL of pure filtered fresh mango juice was then added into the hydrolyzed cassava medium culture according to a volume ratio of 75:25. The medium culture was adjusted to desired pH by using solutions of 1 N of NaOH and 1 N of H₂SO₄. The medium culture was sterilized at 121°C for 20 minutes to avoid contamination and then cooled down to room temperature. The inoculum was prepared by using baker’s yeast incubated in glucose solution for about 8-10 hours under room temperature. 60 mL of inoculum was added to the fermentation medium prior to the start-up.

2.3. Preparation of mango juice

The rejected mango fruits were needed for mango juice preparation. The damaged parts of fruits were removed. The mango fruits were blended into juice by using blender and the juice was filtered by using a filter bag. The pure filtered fresh mango juice was ready for use.

2.4. Sample analysis

A number of samples were taken throughout the experiment for every 2-3 hours of sampling interval. The concentrations of glucose and ethanol were analyzed by using R-Biopharm test kits and UV spectrophotometer at wavelength of 340 nm under room temperature, based on the procedures provided in the manuals of the test kits. The optical density was analyzed by using UV spectrophotometer at a wavelength of 340 nm for cell concentration. Prior to analysis, the sample was diluted to appropriate concentrations so that the range of absorbance range was between 0.1 and 0.4.

3. Modelling and identification

3.1. Batch bioreactor model

Consider a typical batch bioreactor system of ethanol fermentation.

\[
\begin{align*}
\frac{dX}{dt} &= r_x \\
\frac{dS}{dt} &= -r_s \\
\frac{dP}{dt} &= r_p 
\end{align*}
\]  

(1)

The microbial kinetics include 3 components, i.e., the rate of biomass formation \( r_x \), rate of substrate consumption \( r_s \) and rate of product formation \( r_p \), where the state variables \( X, S \) and \( P \) denote the biomass (kg/m³), substrate (kg/m³) and product (kg/m³) concentrations respectively.

3.2. Microbial kinetic model

The Herbert's concept was proposed which assumed that the observed rate of biomass formation \( r_x \) comprised the growth rate \( r_g \) and the death rate of biomass via catabolism, which represents the rate of endogenous metabolism \( r_d \) [9]. The rates of cellular growth \( r_x \) based on the Herbert's concept is

\[
r_x = r_g + r_d 
\]  

(2)
where the growth rate \( r_g \) comprises the effects of the inhibition imposed by high substrate concentration, i.e., Haldane model [10,11] and death rate \( r_d \) are assumed to be as follows

\[
\begin{align*}
  r_g &= \frac{k_1XS}{k_2 + S + k_3S^2} \\
  r_d &= -k_4X
\end{align*}
\]

The rate of substrate consumption \( r_s \) and the rate of product formation \( r_p \) are assumed to be proportional to the biomass growth rate as follows

\[
\begin{align*}
  r_s &= -k_3r_g \\
  r_p &= k_4r_g
\end{align*}
\]

### 3.3. Kinetic model parameters identification

The kinetic model parameter identification can be determined via a nonlinear regression based on the Sum of Square Error (SSE) criterion, which can be used to obtain optimum values for the parameters corresponding to the best model fitting between the experimental observations and model predictions [12]. The formulation is

\[
E(\theta) = \sum_{n=1}^{np} \left[ \frac{(X_n - X_{en})^2}{X_{emax}} + \frac{(S_n - S_{en})^2}{S_{emax}} + \frac{(P_n - P_{en})^2}{P_{emax}} \right]
\]

where \( \theta \) is the vector of kinetic model parameters, which is constrained by bounds within a realistic range, \( \theta = [k_1, k_2 \ldots k_n]^T \). \( X_{en} \), \( S_{en} \) and \( P_{en} \) are the experimental value of concentrations of cell, substrate and ethanol at the sampling time \( n \), while \( X_n \), \( S_n \) and \( P_n \) are the concentration of cell, substrate and ethanol computed by the models at the sampling time \( n \). \( X_{emax} \), \( S_{emax} \) and \( P_{emax} \) are the maximum measured concentrations, which were taken as 4 kg/m\(^3\), 80 kg/m\(^3\) and 20 kg/m\(^3\) respectively; \( np \) is the number of samples.

### 4. Advanced Expanded Microbial Kinetics (EMK) Model

The kinetic parameter \( K_i = [k_1, k_2 \ldots k_n]^T \) is obtained for the proposed Herbert-Haldane model. In general, \( k_i = f_i(x_1, x_2) \), where \( f_i \) is a nonlinear function whose arguments are the inputs \( x_1 \) and \( x_2 \). A statistical-based equation can be developed via the factorial design of experiment. This method was proposed in [13], where an expression is given as follows

\[
k_i = \beta_{i,0} + \beta_{i,1}x_1 + \beta_{i,2}x_2 + \beta_{i,3}x_1x_2
\]

where \( k_i \) denotes the kinetic parameter with the interaction between \( x_1 \) and \( x_2 \) is taken into account via the last term in (8). One of the limitation of this method is that \( \beta_{i,0} \neq k_i \) at the baseline condition, i.e., at \( x_1 = x_2 = 0 \) when \( k_i \) is strongly nonlinear. In other words, the method [13] only applies to a case where \( k_i \) is mildly nonlinear in \( x_1 \) and \( x_2 \). In this paper, the following modification to (8) is proposed so as to force \( \omega_0 = k_i \) for the case where \( k_i \) is strongly nonlinear function of \( x_1 \) and \( x_2 \). For the kinetic parameter \( k_i \), for \( i = 1, 2, \ldots 6 \), we propose a function in the form of

\[
k_i = k_i + e_{i,3}(\beta_{i,1}x_1 + \beta_{i,2}x_2 + \beta_{i,3}x_1x_2)
\]
where $\bar{k}_i$ is the kinetic parameter for baseline (run 0), $\beta_{i,j}$ denotes the model parameters at $j^{th}$ run, and $\epsilon_{i,s}$ is an error describing a function with $x_1$ and $x_2$ as input arguments.

For a given experimental run, the error is calculated as

$$
\epsilon_{i,s} = \frac{k_i - \bar{k}_i}{\beta_{i,1}x_1 + \beta_{i,2}x_2 + \beta_{i,3}x_1x_2} \quad (10)
$$

Let us assume that the error can be expressed by an equation in terms of $x_1$ and $x_2$

$$
\epsilon_{i,s} = \alpha_{i,0} + \alpha_{i,1}x_1 + \alpha_{i,2}x_2 + \alpha_{i,3}x_1x_2 \quad (11)
$$

Therefore, the overall desired response for the estimated kinetic parameter $k_i$ in (9) for $i = 1, 2, \ldots 6$ is obtained by substituting (11) into (9), which can be written in a form of

$$
k_i = \bar{k}_i + a_{i,1}x_1 + a_{i,2}x_2 + a_{i,12}x_1x_2 + a_{i,11}x_1^2 + a_{i,22}x_2^2 + a_{i,112}x_1^2x_2 + a_{i,122}x_1x_2^2 + a_{i,1122}x_1^2x_2^2 \quad (12)
$$

where the parameters in (12) are expressed in terms of the parameters of statistical-based equations in (9) and (11)

$$
\begin{bmatrix}
a_{i,1} \\
a_{i,2} \\
a_{i,12} \\
a_{i,11} \\
a_{i,22} \\
a_{i,112} \\
a_{i,122} \\
a_{i,1122}
\end{bmatrix} =
\begin{bmatrix}
\alpha_{i,0} & \beta_{i,1} \\
\alpha_{i,0} & \beta_{i,2} \\
\alpha_{i,1}+\alpha_{i,2}+\alpha_{i,0} & \beta_{i,1} \\
\alpha_{i,1} & \beta_{i,1} \\
\alpha_{i,2} & \beta_{i,2} \\
\alpha_{i,3}+\alpha_{i,1} & \beta_{i,1} \\
\alpha_{i,3} & \beta_{i,1} \\
\alpha_{i,3} & \beta_{i,3}
\end{bmatrix} \quad (13)
$$

The advantages of this modified EMK model (12) is that, it requires only a small number of experimental runs to obtain a 4th order response of the estimated kinetic parameter.

Based on 2 input parameters $x_1$ and $x_2$, the following steps are proposed to identify the parameters in (13):

- **Step 1:** Perform the factorial design of experiment, which gives $2^2 + 1$ number of experiments, i.e., runs 0, 1, 2, 3, 4 where run 0 indicates the baseline condition. Fit the model parameters to a set of data at each experimental run based on the proposed Herbert-Haldane model given in (2)-(6) in order to obtain the kinetic parameters $\theta = [k_1, k_2, \ldots k_6]^T$. The *fmincon* function in Matlab can be used to conduct the nonlinear regression.

- **Step 2:** Use *rstool* function in Matlab to obtain $\beta_{i,1}, \beta_{i,2}$ and $\beta_{i,3}$ in (9) based on run 1, 2, 3, 4.

- **Step 3:** Calculate the error $\epsilon_{i,s}$ for run 1, 2, 3, 4 using (10).

- **Step 4:** Use *rstool* function to obtain $\alpha_{i,0}, \alpha_{i,1}, \alpha_{i,2}$ and $\alpha_{i,3}$ in (11).

- **Step 5:** Use the relationships in (13) to calculate the model parameters in (12).

4. Results and discussion

By applying the above mentioned steps to kinetic parameters, the values of parameters of (13) for $k_i$, for $i = 1, 2, 3, 4, 5, 6$ are shown in Table 2. Fig. 1 shows the response surface of kinetic parameters of $k_i$, for $i = 1, 2, 3, 4, 5, 6$ based on the advanced EMK model. The figure shows that all of the kinetic parameters are strongly nonlinear function of $x_1$ (pH) and $x_2$ (AR). Due to this strongly nonlinearity, the parameters cannot be predicted using the simple EMK model proposed in [13]. Here, the advanced EMK model has been proposed by using a 4th order response to predict the kinetic parameters more accurately. Of course, an alternative to capturing such a high nonlinearity is using the well-
known neural network model. But it is important to note that, to develop a good neural network model, a lot experimental data is required in order to train the neural model properly. In contrast to the neural network modelling, the advanced EMK model can be developed using much less experimental data.

Table 2. The value of parameters of (13) for $k_i$, for $i = 1, 2, ... 6.$

<table>
<thead>
<tr>
<th></th>
<th>$a_{11}$</th>
<th>$a_{12}$</th>
<th>$a_{111}$</th>
<th>$a_{112}$</th>
<th>$a_{22}$</th>
<th>$a_{122}$</th>
<th>$a_{1122}$</th>
<th>$a_{222}$</th>
<th>$a_{1122}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>-0.7935</td>
<td>-0.6199</td>
<td>3.7921</td>
<td>0.0471</td>
<td>3.3240</td>
<td>5.3244</td>
<td>6.8156</td>
<td>3.3341</td>
<td></td>
</tr>
<tr>
<td>$k_2$</td>
<td>665.7835</td>
<td>597.1470</td>
<td>2175.0011</td>
<td>665.7808</td>
<td>879.6068</td>
<td>1577.8536</td>
<td>1759.2133</td>
<td>879.6065</td>
<td></td>
</tr>
<tr>
<td>$k_3$</td>
<td>-0.6665</td>
<td>5.3546</td>
<td>2.5942</td>
<td>0.3278</td>
<td>-0.6971</td>
<td>-2.6525</td>
<td>0.3301</td>
<td>0.9595</td>
<td></td>
</tr>
<tr>
<td>$k_4$</td>
<td>0.1560</td>
<td>-0.0725</td>
<td>0.5020</td>
<td>-0.3978</td>
<td>-0.2407</td>
<td>0.2540</td>
<td>-0.5462</td>
<td>0.3317</td>
<td></td>
</tr>
<tr>
<td>$k_5$</td>
<td>-0.0237</td>
<td>0.0237</td>
<td>-0.0999</td>
<td>0.0328</td>
<td>0.0328</td>
<td>0.0452</td>
<td>-0.0452</td>
<td>-0.0035</td>
<td></td>
</tr>
<tr>
<td>$k_6$</td>
<td>-0.0700</td>
<td>0.0360</td>
<td>-0.0290</td>
<td>-0.0832</td>
<td>0.0575</td>
<td>-0.0619</td>
<td>0.1206</td>
<td>0.0630</td>
<td></td>
</tr>
</tbody>
</table>

(a) (b) (c) (d)
A very small error (less than $10^{-6}$) is encountered in the predicted kinetic parameters compared to the kinetic parameters obtained based on the Herbert-Haldane model with fixed parameters. For illustrations, Fig. 2 and Fig. 3 show the comparison between EMK model prediction and experimental data for run 1 and run 3 respectively. The bioreactor model using the advanced EMK model provided good fitting to the experimental data of biomass, substrate and ethanol concentrations for run 1 and run 3. In Fig. 3, the advanced EMK model gave a smooth curve in the biomass growth prediction especially in describing the endogenous metabolism behavior at the end of experiment – the decay phase.
5. Conclusions

In this paper, a simple procedure for constructing an advanced EMK model has been presented to predict the kinetic parameters of a highly nonlinear dynamic fermentation process. The EMK model represents a single function of a given microbial kinetic parameter which can account the simultaneous effects of pH and aeration rate. In comparison with a neural network model, the advantage of using this modified EMK model is that it requires only a small number of experimental runs to obtain a 4th order response of estimated kinetic parameter. Thus, the model can be provides a higher accuracy in estimating the kinetic parameter value in a case when it is highly nonlinear function of certain input parameters, e.g., pH and aeration rate. It is worth highlighting that, the advanced EMK model is easy and inexpensive to construct, thus it can be used in bioreactor simulation, optimization and control studies. In future study, the current EMK model will be extended to capturing the simultaneous effects of more than two input parameters, for example, simultaneous effects of pH, aeration rate and medium temperature.

Fig. 3. Comparison between advanced EMK model prediction (solid line) and experimental data (dot) for run 3.

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References


