EFFECT OF HYDRODYNAMICS ON KINETICS OF GLUCONIC ACID ENZYMATIC PRODUCTION IN BUBBLE COLUMN REACTOR

Oxidation of glucose by homogeneous glucose oxidase was performed in rectangular bubble column reactor at 40 °C, ambient pressure and pH of 5.5 while superficial gas (oxygen) velocity was varied in the homogeneous and transition regime in the range of 0.0014–0.0112 m s⁻¹. Effect of superficial gas (oxygen) velocity on the apparent reaction rate and its parameters was determined and it was observed that the apparent reaction rate on the basis of volume of the liquid increased with increasing the superficial gas (oxygen) velocity. The apparent reaction rate was assumed to be in the form of Michaelis-Menten equation and its apparent kinetic parameters were evaluated by the nonlinear regression method.

Keywords: bubble column; kinetics; hydrodynamics; Michaelis-Menten equation; oxygen velocity.

Bubble columns are gas-liquid contactors in which a gas consisting of one or more reactants is distributed into the column by a sparger and reacts with the liquid phase itself or with a component dissolved or suspended in it [1]. With their simple construction, no mechanically moving parts, efficient mixing and low shear stress and good heat and mass transfer properties, these reactors are becoming more popular in biological processes compared with stirred reactors [2,3].

Bioconversion of glucose to gluconic acid is one of the well known processes in the biological industries. Gluconic acid and its salts are important materials used in pharmaceutical, food, textile, detergent, leather, photographic and other biological industries [4]. In fact, gluconic acid and D-gluconolactone are simple dehydrogenation products of oxidation of D-glucose obtained by glucose oxidase (E.C.1.1.3.4) [5-7]. A detailed description of kinetics of gluconic acid production has been published in several papers [8-10]. The mechanism of this bioconversion can be given by three steps:

\[
\text{glucose} + \text{O}_2 \xrightarrow{\text{GOD}} \text{D-gluconolactone} + \text{H}_2\text{O}_2 \quad (1)
\]

\[
\text{H}_2\text{O}_2 \xrightarrow{\text{CAT}} \text{H}_2\text{O} + \frac{1}{2}\text{O}_2 \quad (2)
\]

\[
\text{D-gluconolactone} + \text{H}_2\text{O} \xrightarrow{\text{LAC}} \text{gluconic acid} \quad (3)
\]

where GOD is glucose oxidase, CAT is catalase and LAC is lactonase. The overall reaction can be considered as follows:

\[
\text{glucose} + \frac{1}{2}\text{O}_2 \xrightarrow{\text{GOD,CAT}} \text{gluconic acid} \quad (4)
\]

Nakamura and Ogura [11] proposed the kinetics of the oxidation of glucose by the glucose oxidase from Penicillium amagaskiense. They expressed that semiquinoid intermediates do not affect the reactive mechanism of glucose oxidase. Thereafter, Gibson et al. [5] determined the kinetics and mechanism of glucose oxidase in two different ways, including monometric and stopped flow experiments. They measured the kinetics of glucose oxidase reaction with diverse substrates such as glucose, mannose, xylose and 2-deoxyglucose and concluded that the rate of glucose oxidation is considerably faster than other reactions. Furthermore, they mentioned that the rate of oxidation of glucose depends on oxygen concentration as well as temperature.
Despite sufficient amount of surveys conducted on kinetics of reaction and mass transfer characteristics of immobilized glucose oxidase [12,13], there are rather fewer studies focused on homogeneous glucose oxidase. Nakao et al. [14] investigated mass transfer characteristics and optimal operating conditions for producing gluconic acid with immobilized glucose oxidase in airlift and bubble column reactors. They concluded that bubble column reactor provides higher gluconic acid productivity and lower glucose oxidase activity decay compared to other type of reactors due to its better mass transfer properties. Afterwards, Bang et al. [15] investigated glucose oxidation in a three-phase stirred airlift reactor. They highlighted the influence of oxygen concentration and gas velocity on the reaction rate and concluded that the reaction rate noticeably increases with oxygen concentration in gas phase as well as gas velocity. Furthermore, Klein et al. [16] and Znad et al. [17] demonstrated the positive influence of air flow rate on the reaction rate of gluconic acid production.

Although the effect of temperature on the reaction rate of glucose oxidation has been studied, there are limited surveys dedicated to thorough investigations of the effect of gas velocity on this rate. Accordingly, the aim of this work is to investigate the effect of gas velocity and hydrodynamics of the bubble column on the rate of enzymatic oxidation of glucose by glucose oxidase. The reason for choosing the bubble column reactor is that considerably higher amounts of gluconic acid can be produced, glucose oxidase activity decay is lower, and mass transfer properties are better in this reactor compared to other types of reactors [14]. In the present work, glucose was oxidized by homogeneous glucose oxidase at various gas velocities at 40 °C. Considering the Michaelis-Menten equation, the apparent reaction rate parameters were determined. The results of this work can be used in future studies concerning the modeling of bubble columns used in bioprocesses, taking into account reaction and hydrodynamic parameters.

EXPERIMENTS AND METHODS

Bioreactor set-up

The schematic of the experimental setup used in this work is shown in Figure 1. The rectangular bub-

![Figure 1. Schematic of the experimental set-up.](image-url)
ble column was made of Plexiglas with dimensions of 0.12 m width, 0.7 m height and 0.05 m depth. The oxygen was introduced through a Plexiglas perforated plate sparger located 0.03 m above the base of the reactor. The sparger was rectangular with the dimensions of 0.12 m length and 0.05 m width. It contained 14 orifices with diameter of 0.0006 m and 0.01 m square pitch. The design of the reactor and sparger was based on the findings of Buwa and Ranade [18] who showed that the sparger design has an insignificant effect on the turbulence and flow regime (for this kind of sparger, with this pitch and hole diameter). A rectangular bubble column was utilized in order to improve the accuracy of measuring bubble sizes as in cylindrical bubble column reactors the curvature of the reactor introduces error when determining bubble size through photographic method.

Materials

The D-Glucose monohydrate (99% pure) from Fluka was used as reactant in different concentrations of 0.0555, 0.222, 0.3885 and 0.555 mol/ L. Distilled water was used as solvent in all experiments. Glucose oxidase produced by Aspergillus niger (Sigma-Aldrich Company) fermentation which was claimed to contain 1 mg g⁻¹ of flavine-adenine dinucleotide (FAD) and with activity of 24,800 units g⁻¹ (unit definition: required amount of enzyme to oxidize 1 μmol of glucose to gluconic acid and H₂O₂, per minute at 25 °C and pH 7). The catalase was produced by bovine liver with activity of 3940 units mg⁻¹. Acetate buffer with pH 5.5 was used for adding the glucose oxidase and catalase to the solution of glucose. The physical properties of glucose solution with two enzymes used in this work are listed in Table 1.

Methods

All experiments were carried out at controlled temperature (±0.1 K) and pH value (±0.1) using a Mettler Toledo DL28 titrator using 1 M NaOH. The pH of the solution was maintained at 5.5 (maximum GOD activity) by NaOH, which was automatically added to the solution in order to neutralize gluconic acid. The catalase and glucose oxidase with concentration of 0.0101 g/L were added to the aqueous solution of glucose at various concentrations. Since oxygen was dissolved in the liquid, nitrogen gas was passed through the column to remove the oxygen. The gas was then changed to oxygen and oxygen concentration in the liquid phase was measured by a Mettler Toledo oxygen sensor connecting to a PC and recorded online. Oxygen was introduced into the column through a sparger at superficial velocities of 0.0014, 0.0028, 0.0056 and 0.0112 m/s. All experiments were carried out in the homogeneous and transition regime at atmospheric pressure and 40 °C.

For highlighting the effect of gas hold-up and specific gas-liquid interfacial area on reaction rate, this hydrodynamic parameter were determined by the following equations [1]:

\[
\varepsilon_a = \frac{H_o - H_L}{H_o} \tag{5}
\]

\[
d_{32} = \sum d_i^3 \sum d_i^2 \tag{6}
\]

\[
a = \frac{6\varepsilon_a}{d_{32}} \tag{7}
\]

In order to determine the Sauter mean diameter \(d_3\), the equivalent sphere diameter of bubble \(d_i\) is evaluated by Eq. (8) and photographic method:

\[
d_i = \sqrt[3]{\frac{2E e}{\pi}} \tag{8}
\]

For evaluating the bubble size distribution, the resulting data from Eq. (8) and number of bubbles in each size acquired from photographs were considered. The fraction of each bubble size in the total length of reactor was obtained from the proportion of number of bubbles in each size to the total number of bubbles.

In order to assess the volumetric mass transfer coefficient \(k_L a\), non-stationary or dynamic method was used. Under the assumptions of ideal mixing in gas and liquid phases, constant interfacial area and no significant change in oxygen concentration in the gas phase, the volumetric mass transfer coefficient can be determined from [19]:

\[
\frac{c^* - c_L}{c^* - c_0} = \left( e^{-k_L a \tau} - k_L a \tau_0 e^{-\frac{1}{\gamma}e^{-k_L a \tau_0}} \right) \left( 1 - k_L a \tau_0 \right) \tag{9}
\]

Table 1. Physical properties of glucose solution used at 40 °C

<table>
<thead>
<tr>
<th>Glucose concentration, mol L⁻¹</th>
<th>ρ / kg m⁻³</th>
<th>μ×10⁻³ / Pa s</th>
<th>σ×10⁻³ / N m⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05551</td>
<td>982.6</td>
<td>0.6891</td>
<td>69.79</td>
</tr>
<tr>
<td>0.222</td>
<td>993.7</td>
<td>0.7463</td>
<td>69.64</td>
</tr>
<tr>
<td>0.3885</td>
<td>1000</td>
<td>0.8124</td>
<td>69.47</td>
</tr>
<tr>
<td>0.5551</td>
<td>1017</td>
<td>0.8870</td>
<td>69.30</td>
</tr>
</tbody>
</table>
In Eq. (9), $\tau_p$ represents the response time of the oxygen probe which was evaluated as the time needed to reach 63% of the value finally approached when exposed to a step change in concentration [19,20]. In the present work, this constant can be assessed by transferring the oxygen probe kept in a solution of sodium sulfite for 0.5 min (wherein the oxygen concentration is zero) to another solution saturated with oxygen (which in this work was glucose solution). The response time ($\tau_p$) of the oxygen probe used in this work was evaluated to be 25 s at 40 °C.

Due to the fact that hydrogen peroxide has the inhibition effect on GOD and reaction rate [9,21-23], it was necessary to prevent its production by adding sufficient amount of catalase, thus, no free hydrogen peroxide was observed. In order to determine the reaction rate, the concentration of gluconic acid was measured by titration with NaOH. The rates of gluconic acid production at various gas velocities were evaluated by measuring the slope of lines showing the time courses of gluconic acid production while the zero time was taken after 700 s from the enzyme addition.

**RESULTS AND DISCUSSION**

**Effect of gas velocity**

The quantitative determination of gluconic acid production, evaluated by titration with NaOH, is illustrated in Figure 2 at glucose concentration of 0.0555 mol L⁻¹. The error bars represent standard deviations of the observed values and are presented only for oxygen velocity of 0.0056 m s⁻¹ as an example. The error bars for other oxygen velocities were fairly in the same range and are not displayed to make the figure easy to understand. It can be seen in these figures that variations of gluconic acid concentrations vs. time are linear with the correlation coefficient greater than 0.985. These slopes determine the apparent rate of gluconic acid production. It can be seen that oxygen velocity has a positive effect on producing of gluconic acid and the reaction rate.

The determined reaction rate versus oxygen velocity is illustrated in Figure 3. It can be easily recognized that gas velocity has a positive effect on reaction rate and with increasing gas velocity, the reaction rate increases. The results of this study are in agreement with those in literature [15,16]. Bang et
al. [15] concluded that the reaction rate depended on gas velocity for \( u_G < 1.17 \text{ cm s}^{-1} \) while it was independent of gas velocity for \( u_G > 1.17 \text{ cm s}^{-1} \). In the present work, the range of gas velocity was between 0.14-1.12 cm s\(^{-1}\) which is less than 1.17 cm s\(^{-1}\) and the reaction rate was found to be affected by the gas velocity. Higher oxygen velocity amplifies significantly supply of oxygen into the bioreactor and increases gas hold-up which assists to raise the intensity of mixing and leads to boosting the oxygen transfer rate and increases the reaction rate as a result. Figure 3 also shows that the reaction rate is nearly constant in the range of gas velocity of 0.0028 to 0.0056 m s\(^{-1}\). This trend can be related to the available interfacial area in the reactor. The effect of glucose concentration on the reaction rate can also be comprehended from this figure. It is obvious that with increasing glucose concentration the apparent reaction rate is increased.

Figure 4 demonstrates the bubble size distribution at various gas velocities considered in this work. It can be seen in this figure that the bubbles at 0.0028 m s\(^{-1}\) are slightly smaller than that at 0.0056 m s\(^{-1}\). It is worth mentioning that according to Buwa and Ranade [18] at the gas velocity of 0.0112 m s\(^{-1}\) there was a transition regime in the bubble column reactor. While higher gas velocity provides more bubbles in the reactor, presence of larger bubbles reduces the inter-phase mass transfer area. As a result, it is suggested that gas velocities of 0.0028 and 0.0056 m s\(^{-1}\) provide almost the same concentration of oxygen in the liquid phase. Consequently, the reaction rates at these gas velocities become the same. In fact, below 0.0028 m s\(^{-1}\) the bubbles are small and relatively far separated each other. Therefore, increasing the gas velocity below this limit only increases the number of bubbles (corresponding to increase in inter-phase mass transfer area). At gas velocities higher than 0.0028 m s\(^{-1}\) the number of bubbles becomes so high that they become closer to each other and coalescence of bubbles begins. Although coalescence of bubbles still exists at gas velocities higher than 0.0056 m s\(^{-1}\), the increase in the number of bubbles outweighs the decrease in the interfacial area, thus, the concentration of oxygen in the liquid phase increases with increasing the gas velocity and the reaction rate increases accordingly.

Figure 5 illustrates the effect of glucose concentration on gas hold-up. This figure demonstrates that the gas hold-up decreases with increasing the glucose concentration. In fact, the viscosity of solution increases with increasing the glucose concentration, resulting in formation of larger bubbles. Consequently, the number of bubbles decreases and their rise velocity increases, which makes the gas hold-up decrease. Figure 5 also shows that the gas hold-ups
Figure 4. Bubble size distribution at various gas velocities and glucose concentration of 0.555 mol L⁻¹.

Figure 5. Gas hold-up at various superficial gas velocities as a function of glucose concentration (error bars are standard deviations).
for gas velocities of 0.0028 and 0.0056 m s\(^{-1}\) are reasonably close to each other.

Variation of specific gas-liquid interfacial area with glucose concentration at various gas velocities is shown in Figure 6. It can be seen in this figure that the specific gas-liquid interfacial area decreases with increasing the glucose concentration. In fact, with increasing the glucose concentration, the gas hold-up decreases which results in decreasing the specific gas-liquid interfacial area (see Eq. (7)). Figure 6 also reveals that the specific gas-liquid interfacial area at both gas velocities of 0.0028 and 0.0056 m s\(^{-1}\) are very close. This also can be attributed to close values of gas hold-up and mean bubble size at these velocities.

Figure 7 shows the effect of glucose concentration on volumetric oxygen transfer coefficient. This figure reveals that the mass transfer coefficient decreases with increasing the glucose concentration. This negative effect of glucose concentration on mass transfer coefficient can be attributed to the viscosity of solution. Increasing the glucose concentration increases the viscosity of solution, thus, the turbulent intensity and gas hold-up decrease. As a result, the specific gas-liquid interfacial area decreases and does the oxygen transfer coefficient. It can be seen in Figure 7 that oxygen transfer coefficient for gas velocities of 0.0028 and 0.0056 m s\(^{-1}\) are close. As stated before, the specific gas-liquid interfacial areas for these velocities are significantly close which is the reason for observing almost the same values of oxygen transfer coefficients at these velocities. The experimental results of this work were compared with existing experimental correlations for volumetric mass transfer coefficient. Among the correlations, the correlation proposed by Akita and Yoshida [24]:

\[
\frac{k_a D_c^2}{D_{ab}} = 0.6 \left( \frac{\mu_a}{\rho_a D_{ab}} \right)^{0.5} \left( \frac{gD_c^2 \rho_l}{\sigma} \right)^{0.62} \left( \frac{gD_c^2 \rho_l^2}{\mu_c^2} \right)^{0.31} \epsilon_{G3}^{1.1}
\]

is in satisfactory agreement with the experimental data as demonstrated in Figure 8. The relative disparity between these results with Akita and Yoshida [24] is attributed to difference in dimension of bubble column reactor, kind and diameter of sparger, physical properties of materials and superficial gas velocity.

Since the reaction rate depends on the gas hold-up, the reaction rate should be presented as a function of this hydrodynamic parameter. Therefore, the apparent reaction rate obtained in Figure 2 with the unit of mol/m\(^3\) of liquid/h should be converted into mol/m\(^2\) of interfacial area/h through the following expression:
Figure 7. Effect of glucose concentration on volumetric mass transfer coefficient at various superficial gas velocities (error bars are standard deviations).

Figure 8. Comparing the experimental results with the correlation proposed by Akita and Yoshida [24].
\[
\frac{r'}{m^2 \text{ Interfacial area hr}} = \frac{r \text{ mol to mL}}{m^2 \text{ Liq hr}} \times \frac{(1-e_G)}{\varepsilon_G} \times \frac{1}{m^2 \text{ Gas a}} \times \frac{a_G}{m^2 \text{ Interfacial area}} = a_L
\]

where \( a = a_G e_G \) and \( a_L = a/(1-e_G) \). Converted reaction rate against glucose concentration is illustrated in Figure 9 at various gas velocities. It can be seen in this figure that the reaction rate decreases with increasing the gas velocity at various glucose concentrations. The reason for this change in trends of \( r' \) with gas velocity is related to gas hold-up and specific gas-liquid interfacial area \( a_G \) included in the converted reaction rate. With increasing the gas velocity, gas hold-up \( (\varepsilon_G) \) and specific gas-liquid interfacial area \( a_G \) increase and the reaction rate decreases when the rate \( r \) is almost constant irrespective of the superficial gas velocity, i.e., when \( r \) is nearly equal to the chemical reaction rate.

The parameter \( a_L \) is in fact the specific interfacial area per unit volume of the liquid and is a function of the properties of gas and liquid. The variation of \( a_L \) with glucose concentration is almost the same as that of \( a \), which is shown in Figure 6. Therefore, it is related to the concentration of glucose in the solution. The parameter \( a_L \) is a strong function of the glucose concentration at low gas velocity while with increasing the gas velocity becomes almost independent of the glucose concentration. The reason for such a trend can be explained by the change in the physical properties of the solution with glucose concentration. The viscosity of the solution increases with increasing the concentration of glucose. As a result, larger bubbles are formed at higher glucose concentration which reduces the gas holdup and the specific interfacial area. This is the reason for observing the decreasing trend in \( a_L \) with increasing the concentration of glucose. It is worth mentioning that the same effect of gas velocity on the reaction rate has been already pointed out by Bang et al. [15]. This factor can explain dependence of the reaction rate on the oxygen velocity at low superficial gas velocities and its independence at high gas velocities.

**Evaluation of apparent kinetic parameters**

The mechanism of glucose oxidation by GOD was proposed by Nakamura and Ogura [11] and in more details by Gibson et al. [5]. They proposed that oxidation of glucose consists of the following two steps with four apparent kinetic parameters:

\[
E_{ox} + G \xrightarrow{k_1} E_{red}, \text{Glu} \xrightarrow{k_2} E_{red} + \text{Glu}
\]

\[
E_{red} + O_2 \xrightarrow{k_3} E_{ox}, \text{H}_2\text{O}_2 \xrightarrow{k_4} E_{ox} + \text{H}_2\text{O}_2
\]

Duke et al. [25] developed a rate equation for this mechanism with the hypothesis of steady state

![Figure 9. Reaction rate per unit interfacial area vs. glucose concentration at various gas velocities (error bars are standard deviations).](image-url)
condition. With the assumption that the observed rate \( r \) is the chemical reaction rate, the proposed rate equation is expressed as follows:

\[
\frac{C_{E,j}^0}{r} = \frac{1}{k_c C_G} + \frac{1}{k_2 + k_3 C_{O_2} + \frac{1}{k_4}} \tag{14}
\]

According to Beltrame et al. [26], the following composite coefficient \( k_c \):

\[
\frac{1}{k_c} = \frac{1}{k_2} + \frac{1}{k_3 C_{O_2} + \frac{1}{k_4}} \tag{15}
\]

can be substituted into Eq. (14) and the equation of apparent reaction rate would be converted into the Lineweaver-Burk form:

\[
\frac{1}{r} = \frac{1}{C_{E,j}^0} \left( \frac{1}{k_c C_G} + \frac{1}{k_c} \right) \tag{16}
\]

or the analogous form of Michaelis-Menten equation:

\[
r = \frac{k_c C_{E,j}^0 C_G}{k_c / k_1 + C_G} \tag{17}
\]

In order to determine the apparent kinetic parameters of Michaelis-Menten rate equation (\( k_c \) and \( k_1 \)), a least square nonlinear regression technique was utilized. Calculated rate constants are given in Table 2. It should be noted that the results in Table 2 are valid only for the limited ranges of enzyme concentration, partial pressure of oxygen and glucose concentration employed in this study. For comparison of these results with other publications, it is necessary to compromise the resulting units with the reported units. For attaining this purpose, the enzyme concentration should be reported in molar. Thereby, the results should be divided by \( 1.27 \times 10^{-6} \), as our enzyme contains 1 mgFAD g\(^{-1}\) GOD that is \( 1.27 \times 10^{-6} \) molFAD g\(^{-1}\) GOD. The converted values are illustrated in Table 2. These apparent kinetic parameters are compared with those reported in literature [25,26] as described below. Figure 10 demonstrates the variation of apparent reaction rate

### Table 2. Evaluation of \( k_c \) and \( k_1 \) for different oxygen velocity examined with two kinds of units

<table>
<thead>
<tr>
<th>Gas velocity, m s(^{-1})</th>
<th>( k_c )</th>
<th>( k_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol g(^{-1}) h(^{-1})</td>
<td>s(^{-1})</td>
</tr>
<tr>
<td>0.0014</td>
<td>0.0758</td>
<td>16.58</td>
</tr>
<tr>
<td>0.0028</td>
<td>0.1351</td>
<td>29.56</td>
</tr>
<tr>
<td>0.0056</td>
<td>0.1424</td>
<td>31.16</td>
</tr>
<tr>
<td>0.0112</td>
<td>0.2015</td>
<td>44.08</td>
</tr>
</tbody>
</table>

![Figure 10. Parity plot of calculated apparent reaction rate vs. experimental data.](image-url)
(calculated based on the constants reported in Table 2) as a function of superficial gas velocity and compared with experimental data. It can be seen in this figure that the calculated results are in satisfying agreement with the experimental data with correlation coefficient greater than 0.9436.

Beltrame et al. [26] estimated $k_c$ and $k_1$ in the range of 0-30 °C. At 30 °C, they reported 0.6606 mol g⁻¹ h⁻¹ and 28.10 L g⁻¹ h⁻¹ for $k_c$ and $k_1$, respectively. According to their results, larger values of $k_c$ and $k_1$ would be expected at 40 °C. However, $k_c$ and $k_1$ in this study are smaller than those reported by Beltrame et al. [26]. One possible reason for this difference is oxygen concentration which in this work is considerably less than that reported by Beltrame et al. [26]. In their research, $C_0$ was equal to 1.18×10⁻⁷ mol L⁻¹ while in this work $C_0$ was between 4.27×10⁻⁵–1.37×10⁻⁴ mol L⁻¹. As $k_c$ is related to steps containing the oxidation of reduced enzyme, lower values resulted in this study compared to those in Beltrame et al. [26]. The lower amount of oxygen dissolved in the liquid phase leads to increase in the amount of reduced enzyme which is converted to $E_{ox}.H_2O_2$ (Eq. (13)) and apparent kinetic parameter related to this step is decreased as a consequence. It is worth noting that the reaction rate is a weak function of oxygen velocity. In accordance with Beltrame et al. [26] and results of this work, it can be concluded that the reaction rate depends more on temperature than oxygen velocity. The values of $k_1$ are between 537-3884 s⁻¹ while in the literatures, those are fairly higher. Gibson et al. [5] reported 2100 s⁻¹ for 0 °C and 16000 s⁻¹ for 38 °C. It is followed by Duke et al. [25] who disclosed 3700 s⁻¹ at 0 °C and 23800 at 30 °C. These discrepancies between the results are related to an assumed enzyme concentration and activity which was mentioned about 10⁻⁸ mol L⁻¹ [25] as well as the oxygen transfer limitation. However, the difference is within one order of magnitude which seems plausible.

**CONCLUSIONS**

The effect of oxygen velocity on the apparent reaction rate of oxidation of glucose by homogeneous glucose oxidase was investigated. It was observed that increasing the gas velocity results in increasing the apparent reaction rate due to higher oxygen transfer from gas phase to liquid phase. At two gas velocities of 0.0028 and 0.0056 m s⁻¹ the apparent reaction rates are noticeably close due to the similarity of the gas hold-ups, specific gas-liquid interfacial areas and the volumetric oxygen transfer coefficients in these two velocities. To emphasize effects of the hydrodynamic parameters on the apparent reaction rate, the apparent reaction rate was expressed on the basis of the available interfacial area which includes gas hold-up and specific interfacial area. With assuming the apparent reaction rate in the form of Michaelis-Menten equation, a satisfactory agreement between the experimental and calculated apparent reaction rates was observed. The apparent constants of the Michaelis-Menten equation were determined by the nonlinear regression method.

**Nomenclature**

- $a_G$: specific gas-liquid interfacial area per unit gas volume (m⁻¹)
- $a_L$: specific gas-liquid interfacial area per unit liquid volume (m⁻¹)
- $a$: specific gas-liquid interfacial area based on the dispersion volume (m⁻¹)
- CAT: catalase
- $c^*$: equilibrium dissolved oxygen concentration (mol L⁻¹)
- $C_{e,t}$: initial total enzyme concentration (g L⁻¹)
- $C_G$: glucose concentration (mol L⁻¹)
- $c_L$: dissolved oxygen concentration for physical oxygen absorption (mol L⁻¹)
- $c_O$: dissolved oxygen concentration for glucose oxidation (mol L⁻¹)
- $C_O$: equilibrium dissolved oxygen concentration for glucose oxidation (mol L⁻¹)
- $c_{o,i}$: initial dissolved oxygen concentration for physical oxygen absorption (mol L⁻¹)
- $d_i$: sphere equivalent diameter (m)
- $d_{32}$: mean bubble size (Sauter mean diameter) (m)
- $E$: major axis of the ellipsoid (m)
- $e$: minor axis of ellipsoid (m)
- $E_{ox}$: oxidized enzyme
- $E_{red}$: reduced enzyme
- $G$: glucose
- GOD: glucose oxidase
- $H_G$: dispersion height (m)
- $H_L$: static liquid height (m)
- $k_c$: kinetic parameter of Michaelis-Menten equation (mol g⁻¹ h⁻¹)
- $k_1$: kinetic parameter of Michaelis-Menten equation (L g⁻¹ h⁻¹)
- $k_2$: kinetic parameter related to glucose oxidation (mol g⁻¹ h⁻¹)
- $k_3$: kinetic parameter related to glucose oxidation (L g⁻¹ h⁻¹)
- $k_4$: kinetic parameter related to glucose oxidation (mol g⁻¹ h⁻¹)
$k_a$ volumetric oxygen transfer coefficient (s$^{-1}$)

LAC lactonase

\( r \) apparent rate of gluconic acid production per unit liquid volume (mol L$^{-1}$ h$^{-1}$)

\( r' \) apparent rate of gluconic acid production per unit interfacial area (mol m$^{-2}$ h$^{-1}$)

\( t \) time (s)

\( u_G \) superficial gas (oxygen) velocity (m s$^{-1}$)

\( \varepsilon \) gas hold-up

\( \tau_p \) response time of the oxygen probe (s)

\( G \) gas

\( L \) liquid

Greek letter

\( \varepsilon_G \) gas hold-up

\( \tau_p \) response time of the oxygen probe (s)

Subscripts

\( G \) gas

\( L \) liquid

REFERENCES


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NAUČNI RAD

UTICAJ HIDRODINAMIKE NA KINETIKU ENZIMSKE PRODUKCIJE GLUKONSKE KISELINE U BARBOTAŽNOJ KOLONI

Oksidacija glukoze slobodno suspendovane glukozo oksidaze je izvršena u pravougaonoj barbotažnoj koloni na 40 °C, atmosferskom pritisku i pH 5,5, dok je površinska brzina kiseonika varirana u homogenom i prelaznom režimu u opsegu 0,0014–0,0112 m/s. Određen je uticaj površinske brzine kiseonika na prividnu brzinu reakcije i njene parametre. Uočeno je da se prividna brzina reakcije po jedinici zapremine povećava sa povećanjem površinske brzine kiseonika. Pretpostavljeno je da prividna brzina reakcije sledi Michaelis-Menten-ovu jednačinu, čiji su parametri izračunati metodom nelinearne regresije.

Ključne reči: barbotažna kolona, kinetika, hidrodinamika, Michaelis-Menten-ova jednačina, površinska brzina kiseonika.