**Note**

**Kinetic Analysis of Lactulose Production from Lactose in Subcritical Aqueous Ethanol**

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Production of lactulose from lactose in subcritical aqueous ethanol was analyzed through a series of kinetic experiments. Assuming first-order reaction kinetics for both the reversible isomerization between lactose and lactulose and the hydrolysis of these disaccharides, the rate constants for these processes were estimated from our previous results (Soisangwan et al., in press) using the concentration–time integrals method. In subcritical water, the rate constants for the hydrolysis of lactose and lactulose to their constituent monosaccharides were found to be greater than the rate constant for the isomerization of lactose to lactulose. In subcritical aqueous ethanol, on the other hand, the rate constant for this isomerization reaction was found to be greater than the rate constants for both of the hydrolysis processes. The activation energies for the isomerization of lactose to lactulose and its reverse reaction were determined as 105 and 153 kJ/mol, respectively.

Keywords: lactulose, subcritical fluid, isomerization, concentration–time integrals method

**Introduction**

The keto-disaccharide lactulose, consisting of fructose and galactose joined by a β-1,4-glycosidic linkage (Aït-Aissa and Aïder, 2014), has received significant attention in the food and pharmaceutical industries as a result of its reported health benefits (Bouhnik et al., 2004; Schumann 2002; Wesselinus-De Casparis et al., 1968; Dhiman et al., 2002; Liehr et al., 1980). Lactulose is currently produced by isomerization of lactose in an alkaline medium via the Lobry de Bruyn-Alberda van Ekenstein transformation (Aïder and de Halleux, 2007; Zokaee et al., 2002). However, as a result of the considerable amounts of undesirable side products usually generated using this method, expensive downstream processing is often required (Panesar and Kumari, 2011). An alternative method for the production of lactulose is an enzymatic β-galactosidase- or glycosidase-mediated transgalactosylation reaction (Aït-Aissa and Aïder, 2014). While this production route has many advantages (Wang et al., 2013), the high cost of enzymes restricts its application (Wang et al., 2015).

In the past, we have shown that subcritical aqueous ethanol catalyzes effectively the isomerization of aldo-saccharides to their corresponding keto-saccharides (Gao et al., 2016a; Gao et al., 2015b; Gao et al., 2015b; Gao et al., 2016). This method using aqueous ethanol has several advantages, such as its one-step execution, use of an uncomplicated reactor system, and the easy recovery of ethanol after treatment. Several types of keto-disaccharides, including lactulose (Gao et al., 2016) and cellobiulose (Soisangwan et al., 2016) can be produced from the corresponding aldo-disaccharides by isomerization in subcritical aqueous ethanol. Recently, we...
examined the effect of varying the concentration of ethanol in the reaction solvent and the reaction temperature on the isomerization of lactose to lactulose in subcritical aqueous ethanol (Soisangwan et al., in press). However, the kinetics for the isomerization reaction of lactose to lactulose in subcritical aqueous ethanol has not been reported to date.

In this context, we build on our previous results (Soisangwan et al., in press) and analyze the isomerization of lactose to lactulose in subcritical aqueous ethanol through a kinetic analysis.

Kinetic analysis

Figure 1 shows the assumed mechanism for the production of lactulose from lactose in subcritical aqueous ethanol. In this mechanism, lactose is reversibly isomerized to lactulose, and both disaccharides can be hydrolyzed simultaneously to their constituent monosaccharides. Based on our previous works on isomerization (Soisangwan et al., 2016) and degradation of sugars (Khuwijitjaru et al., in press; Oomori et al., 2004) in subcritical water, first-order kinetics for all steps were assumed. The reaction rates for the formation of lactose and lactulose are given by Eqs. (1) and (2), respectively, where \( C_A \) and \( C_K \) indicate the respective concentrations of lactose and lactulose, \( k_i \) (\( i = 1, -1, 2, \text{ or } 3 \)) is the rate constant, and \( \tau \) is the residence time in a tubular reactor.

\[
\frac{dC_A}{d\tau} = -(k_1 + k_2)C_A + k_1 C_K \quad \text{······Eq. 1}
\]

\[
\frac{dC_K}{d\tau} = k_1 C_A - (k_{-1} + k_2)C_K \quad \text{······Eq. 2}
\]

In order to estimate the rate constants for the processes shown in Eqs. (1) and (2), we employed the concentration−time integrals method, first proposed by Mucientes and de la Peña (2009) for the study of parallel-consecutive first-order reactions with a reversible step. Eq. (1) can be solved by integrating between the limits (0, \( C_{A0} \)) and \( (\tau, C_A) \) for residence time and lactose concentration, to give Eq. (3).

\[
-\int_{C_{A0}}^{C_A} dC_A = (k_1 + k_2) \int_{0}^{\tau} C_A d\tau - k_{-1} \int_{0}^{\tau} C_K d\tau \quad \text{······Eq. 3}
\]

Equation (3) can be rewritten as:

\[
\frac{C_A - C_{A0}}{C_{A0}} = (k_1 + k_2) \int_{0}^{\tau} C_A d\tau - k_{-1} \int_{0}^{\tau} C_K d\tau \quad \text{······Eq. 4}
\]

where

\[
\theta_{A,\tau} = \int_{0}^{\tau} \frac{C_A}{C_{A0}} d\tau \quad (i = A \text{ or } K) \quad \text{······Eq. 5}
\]

Similarly, Eq. (2) can be rewritten to give Eq. (6):

\[
\frac{C_K}{C_{A0}} = k_1 \theta_{A,\tau} - (k_{-1} + k_2) \theta_{K,\tau} \quad \text{······Eq. 6}
\]

The concentration-time integrals \( \theta_i \), at different times were approximated by trapezoidal rule using available data points. Then, the rate constants were estimated using the Solver tool implemented in Microsoft® Excel 2010. This tool employs a non-linear regression technique to adjust the relevant equation parameters to minimize the sum of the squared residuals, i.e., the difference between the experimental and the predicted concentration-time data.

Results and Discussion

Figure 2 shows the changes in the quantity of lactose remaining in the reaction and the quantity of lactulose produced at various ethanol concentrations (% (w/w)) at 200°C (data taken from Soisangwan et al., in press). The rate constants for the reactions shown in Fig. 1 were estimated using the concentration-time integrals method. The reaction profiles for the consumption of lactose and production of lactulose shown in Fig. 2 were constructed using the estimated rate constants. We found that the concentrations of lactose and lactulose predicted using the concentration-time integrals method were in good agreement with the experimental data. These results indicate the adequacy of the proposed kinetic models. To investigate the relationship between the fraction of ethanol in the reaction solvent and the rates of the various reaction pathways, the rate constants obtained for the isomerization and decomposition of lactose at 200°C are plotted against the concentration of ethanol used in the reaction (Fig. 3). In subcritical water (0% (w/w) ethanol), the rate constants for the hydrolysis of lactose and lactulose to their constituent monosaccharides, represented as \( k_1 \) and \( k_{-1} \), were greater than the reaction constant for the isomerization of lactose to lactulose, \( k_i \). This lower relative value for the isomerization constant \( k_i \) can be attributed to the high ion product of water, which is known to accelerate the hydrolysis of disaccharides (Oomori et al., 2004). In contrast, the addition of ethanol to subcritical water led to an increase in the rate constant \( k_i \) which was found to be greater than \( k_1 \) and \( k_2 \) at 20%, 40%, and 60% (w/w) of ethanol. The value of \( k_i \) increased about 6.7 times when using 60% ethanol compared with pure water. This observed enhancement in the formation of lactulose can be ascribed to ethanol-mediated dilution, which decreases the rate of disaccharide hydrolysis (Gao et al., 2014). In this study, we found that the rate constant of lactose degradation \( (k_i) \) also increased with the ethanol concentration, while the rate constant of lactulose degradation \( (k_i) \) showed insignificant changes at 0 – 40% ethanol but rapidly decreased at 60% ethanol. The reversed isomerization rate constant \( (k_i) \) also showed an increasing
trend with ethanol concentration, even though the value at 20% ethanol was unusually high. Moreover, as a result of the strongly basic character of the ethoxide ion existing in subcritical ethanol, the reaction process might be similar to the alkaline isomerization of lactose (Vuorinen and Sjöström, 1982; Buncel et al., 2004; Aider and de Halleux, 2007). Nevertheless, the use of ethanol as a reaction medium is inexpensive, safe, and sustainable owing to its convenient removal (Sheldon, 2005; Kerton and Marriott, 2013).

The effect of reaction temperature on the reaction rates was also examined using our previous results in 60% (w/w) subcritical aqueous ethanol (Fig. 4) (Soisangwan et al., in press). The rate constants at different temperatures were estimated using the same method as in Fig. 2. Specifically, the effect of varying the temperature on the rate constants was probed by plotting the $k_i$ values against the reciprocal of absolute temperature (Fig. 5). The Arrhenius plots shown in Fig. 5 demonstrated that $k_i$ values tend to increase with increasing temperature. However, only $k_1$ and $k_2$ show temperature dependence on the Arrhenius behavior with a high correlation coefficient ($R^2 = 0.99$ and 0.95, respectively). The activation energies for the reaction processes driven by rate constants $k_1$ and $k_2$ were evaluated to be 105 and 153 kJ/mol, respectively. This indicated that the degradation process ($k_2$) was more sensitive to temperature than the isomerization process ($k_1$) of lactose; therefore, at temperatures above 200°C, lactose was converted to its constituent monosaccharides to a higher extent than to lactulose. In addition, the rate constant of lactulose degradation ($k_3$) also markedly increased at 200°C, indicating that a high level of undesirable products was obtained. Therefore, it was not suitable for producing lactulose at this temperature.

A kinetic competition between the forward ($k_1$) and reverse ($k_{-1}$) isomerization processes confirms the importance of ethanol concentration and temperature on the rate constants. Using a series
of kinetic experiments, we found that the rate constant for the forward isomerization process, $k_1$, was larger than that for the reverse reaction, $k_{-1}$, at high treatment temperatures and ethanol concentrations. These results suggest that both an increase in the ethanol concentration and temperature promotes the isomerization of lactose to lactulose. However, the use of higher ethanol concentrations and temperatures were limited due to the low solubility of lactose and facile decomposition of both lactose and lactulose during heating.

**Conclusions**

The concentration-time integrals method was employed efficiently for the analysis of the kinetic parameters governing the conversion of lactose to lactulose in subcritical aqueous ethanol. The rate of the isomerization of lactose to lactulose was promoted by both an increase in the proportion of ethanol in the reaction solvent and the treatment temperature.

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**References**


