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Mixing Effects on the Kinetics of Enzymatic Hydrolysis of Lignocellulosic Sunn hemp fibres for Biofuel Production

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Highlights

- Non-edible Lignocellulosic Sunn hemp fibre is used as substrate for enzymatic hydrolysis
- Maximum yields of reducing sugar (65%) and glucose (60%) are obtained at no mixing
- The nature of product inhibition is experimentally determined to be non-competitive
- Kinetic parameters (K_M, V_{Max}, K_i) for cellulase enzyme measured at various mixing speeds

1. Introduction

Lignocelluloses from non-food crops are considered the most important feedstocks for biofuel production since they do not conflict with food sources. We use a new substrate – non-edible lignocellulosic Sunn hemp fibres, comprising of lignin ($10.32\% \pm 0.84$), hemicelluloses ($10.05\% \pm 0.92$) and cellulose ($75.6\% \pm 2.27$) – to produce biofuel through delignification, enzymatic hydrolysis and microbial fermentation. Delignification separates the lignin from hemicelluloses and celluloses. Enzymatic hydrolysis breaks the β-1-4 glycosidic bonds of the cellulose and the hemicellulose to release their monomeric sugars, and microbial fermentation converts the monomeric sugars to bioethanol. Enzymatic hydrolysis of cellulose extracted from lignocellulosic Sunn hemp fibres is performed using cellulase enzyme at various mixing speeds and substrate loadings. This paper explores the effects of mixing and mass transfer on the kinetics of enzymatic hydrolysis for bioethanol production. The nature of the product inhibition is experimentally determined and the kinetic parameters (K_M, V_{Max}, K_i) are measured at various mixing speeds. Algebraic expressions quantifying the effects of mass transfer on the enzyme-substrate kinetics of hydrolysis are presented.

2. Methods

The experiments are carried out with cellulose concentrations of 10, 20, 30, 40, 50 mg/ml, in 10 ml of 0.1 M sodium acetate buffer solution at a pH of 5.0 in 100 ml conical flasks, at 50°C in an incubator shaker. The mixing speeds are varied as 0, 50, 100, and 150 rpm, and the optimum enzyme:substrate ratio is obtained as 1:15. Samples (200µl) are collected in microcentrifuge tubes at regular time-intervals during 72 hours of enzymatic hydrolysis. The concentrations of reducing sugar and glucose are measured using Dinitrosalicylic Acid (DNS) and Glucose oxidase-peroxidase (GOD-POD) assays, respectively. The kinetic parameters (K_M, V_{Max}, K_i) are calculated for various mixing speeds using standard graphical methods [1].

3. Results and discussion

The effects of mixing on the yields of reducing sugar and glucose are measured during enzymatic hydrolysis. The initial rate of product formation is higher in case of continuous mixing than in no mixing. In the presence of mixing, the reaction is enhanced by convective mass transport between the enzyme and substrate and the boundary layer thickness around the cellulose particles is small. Therefore, mixing reduces the volume of diffusive zone in the reactor and helps the enzyme reach the surface of the cellulose particles across thin diffusion layers [1].

However, as the reaction progresses, the soluble products (reducing sugars) start to accumulate in the diffusion layer. After a certain time, the products – glucose (monomer) and cellobiose (dimer) – accumulated in the boundary layer significantly inhibit the reaction and reduce the product formation. In case of no (convective) mixing, the transport is purely diffusion-driven, and therefore, the product inhibition remains localized in the diffusion layer around the particles. In case of continuous mixing, rapid convective transport uniformly mixes the inhibiting products (glucose and cellobiose) across the reactor, thus "globalizing

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inhibition". Therefore, no mixing (0 rpm) produces higher yields of reducing sugars (65%) including glucose (60%) at the end of 72 hours of enzymatic hydrolysis than convective mixing (50 rpm and above).

The type of product inhibition of cellulase enzyme on cellulose (obtained after delignification of Sunn hemp fibres) during enzymatic hydrolysis is obtained by plotting the inverse of the reaction rate (= time-rate of change of reducing sugar concentration) versus the inverse of substrate concentration at various times. The Michaelis constant (K_M) is calculated from the point of intersection of the different best-fit straight lines corresponding to various time points, while the maximum reaction rate (V_{max}) is calculated by extrapolating the curve of apparent reaction velocity (V'_{max}) versus time to $t=0$. All best-fit straight lines converge on the x-axis and the apparent maximum reaction rate (V'_{max}) decreases with time, both suggesting that the nature of the product inhibition is non-competitive. K_M is obtained as 61.75, 45, 35 and 19 mg/ml, V_{max} is obtained as 0.949, 0.846, 0.711, and 0.519 mg/ml/min, for mixing speeds of 0, 50, 100, and 150 rpm, respectively.
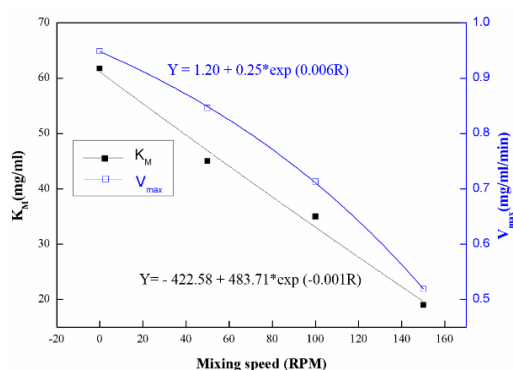

Figure 1. Exponential decay of K_M and V_{max} at various mixing speeds (rpm) for cellulase enzyme.
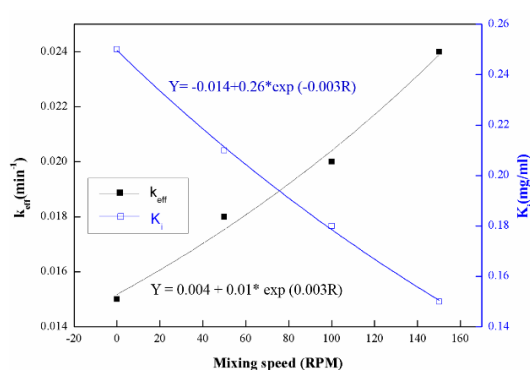

Figure 2. Effective first order reaction rate (k_{eff}) and glucose inhibition (K_i) constants at various mixing speeds.

The experimental values of V_{max} and K_M obtained for four different mixing speeds are plotted in figure 1. Algebraic expressions are developed for V_{max} and K_M as exponential functions of mixing speeds to quantify the effect of mass transfer on reaction kinetics. Figure 2 shows that the effective first order rate constant [3] ($k_{eff}=V_{max}/K_M$) increases and the glucose inhibition constant (K_i) decreases with increasing mixing speed. As the mixing speed increases, mass transfer effects (disguise) on kinetics decreases, resulting in higher k_{eff}, which is obtained as 0.015, 0.018, 0.020, and 0.024 min^{-1}, for 0, 50, 100, and 150 rpm, respectively.

The glucose inhibition constant (K_i) is obtained from the point of intersection of all best-fit straight lines on x-axis while plotting the inverse of the reaction rate versus the glucose (product) concentration for various substrate loadings. The non-competitive product inhibition equation [4] shows that the glucose inhibition constant is directly proportional to the reaction rate. As discussed above, higher convective mixing reduces the reaction rate by increasing (and "globalizing") product inhibition, thereby decreasing K_i, which is obtained as 0.25, 0.21, 0.18, and 0.15 mg/ml, for 0, 50, 100, and 150 rpm, respectively.

4. Conclusions

The type of product inhibition in cellulase-mediated hydrolysis of Sunn hemp fibres is identified as non-competitive. The kinetic parameters (K_M, V_{max}, K_i) decrease exponentially while k_{eff} increases exponentially with mixing speed. Thus, mass transfer limitations exert significant influence on the kinetics of enzymatic hydrolysis. However, the mass transfer limited asymptote of no mixing maximizes the yields of reducing sugars (65%) and glucose (60%) since convective mixing increases the product inhibition across the reactor.

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Keywords
"Lignocellulosic Biofuel", "Enzymatic hydrolysis", "Mixing", "Kinetics".