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Kinetics of heterogeneous biomass hydrolysis by commercial cellulases: effect of biomass pretreatment

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Highlights

- The effect of delignification process on hydrolysis kinetics was assessed
- The harshest pretreatment of biomass led to faster enzymatic hydrolysis
- Assessed kinetics provided quantitative tools for enzymatic hydrolysis reactor design

1. Introduction

Within the framework of lignocellulose biorefinery, main issues related to the upstream processes include the drastic temperature and pressure usually required for efficient delignification of the biomass and the cost of the cellulase enzymes used to obtain monomeric sugars from cellulose hydrolysis. Several studies address the optimization of lignin removal with novel pretreatment (e.g. green solvents) with respect to the resulting sugar yields from enzymatic hydrolysis of the pretreated biomass performed at fixed conditions [1]. Among studies related to characterization of enzymatic hydrolysis, the use of semi-mechanistic models have been adopted to describe the kinetics of heterogeneous conversion of cellulose from lignocellulosic biomass into glucose [2, 3]. To investigate the effect of biomass composition resulting from different chemical and enzymatic delignification pretreatments, kinetic characterization of commercial cocktail Cellic CTec2 (Novozymes) has been carried out adopting apple industrial residues pretreated with NaOH, HCl and laccases.

2. Methods

Biomass pretreatment

Alkaline and acid: The reaction was performed using 1:10 biomass to solvent ratio (dry apple residue: 2% NaOH/HCl) and by keeping the samples in autoclave for 30 min, at 1 atm and 121 °C [4].
Enzymatic: The reaction was performed at 28 °C, 1:10 biomass to solvent ratio (dry apple residue: buffer Sodium citrate pH 5, laccase 10 U/g raw biomass), and mixing the samples at 150 rpm for 24 h.

Enzymatic hydrolysis

Tests were performed in 250 mL overhead stirred batch reactor at 50 °C and with 100 mL of 50 mM Sodium citrate buffer pH 4.8. The concentration of glucose was assessed by enzymatic kit (assay kit K GLU HK 110A, Megazyme). Short term tests (1 h) were performed with solid loading between 1 and 12.0 %w/v and fixed enzyme concentration of 290 FPU/$L_{solution}$ [5]. Alternatively, enzyme concentration was ranged between 5 and 80 FPU/$g_{cellulose}$ [5] and solid loading was fixed at 10 %w/v. Long term assays (72 h) were performed at fixed enzyme concentration of 828 FPU/L and solid loading ranging between 5 and 10 %w/v [5].

Data analysis

Both commercial software (SigmaPlot 10.0) and in-house built MATLAB code were used to regression of experimental data through pseudo-homogeneous Michaelis–Menten (MM), modified MM and Chrastil's kinetic models according to Pratto *et al.* [5].

3. Results and discussion

The most effective delignification was obtained with alkaline pretreatment as well as the lower biomass recovery (available glucan for enzymatic hydrolysis). Fig.1 shows the experimental data from short and long term tests and, as an example, the resulting regression with MM model. Tab.1 reports the kinetic parameters related to the three adopted kinetic models. The faster rate of hydrolysis was obtained for the pretreated biomasses having lower lignin content, that is the alkaline pretreated biomass.

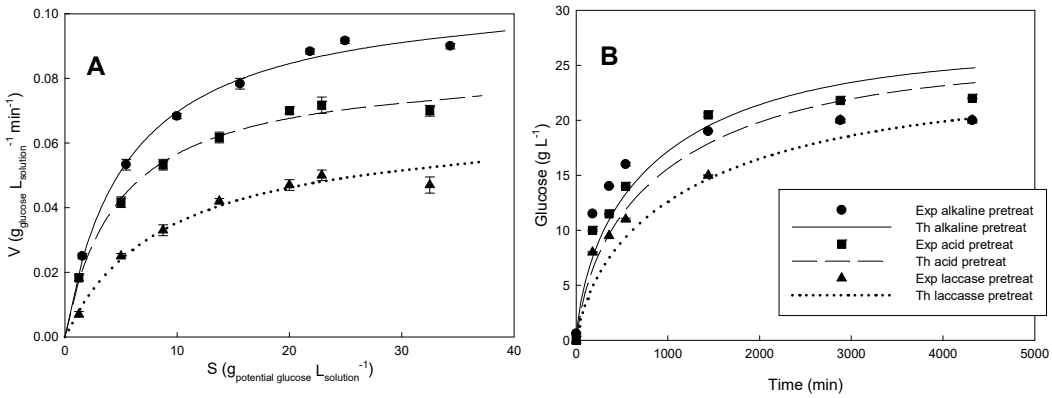

Figure 1. Glucose production rate (A) from short term tests and glucose concentration time course from long term tests (B). Lines show regression with (A) simple MM model and (B) product inhibition MM model.

Biomass pretreatment	Glucan (%wt)	Lignin (%wt)	MM with product inhibition			Modified MM with product inhibition			Chrastil's model	
			V_{max} (g L^{-1} min^{-1})	K_M (g L^{-1})	K_i (g L^{-1})	V_{Emax} (g L^{-1} min^{-1})	K_e (g L^{-1})	K_{Ei} (g L^{-1})	k' (g L^{-1} min^{-1})	n
NaOH	26	5	$0.108\pm3\cdot10^{-3}$	5.5±0.6	0.7±0.2	$0.335\pm8\cdot10^{-3}$	7.6±0.4	0.76±0.19	$1.6\cdot10^{-4}\pm8\cdot10^{-5}$	$0.22\pm5\cdot10^{-2}$
HCl	25	9	$0.084\pm3\cdot10^{-3}$	4.8±0.6	0.67±0.14	$0.297\pm5\cdot10^{-3}$	8.3±0.3	0.66±0.13	$4.5\cdot10^{-4}\pm5\cdot10^{-5}$	$0.42\pm3\cdot10^{-2}$
Laccases	22.5	14.5	$0.067\pm6\cdot10^{-3}$	9±2.1	1.12±0.2	$0.243\pm1.4\cdot10^{-2}$	9.7±1.2	0.57±0.08	$3.9\cdot10^{-4}\pm3\cdot10^{-5}$	$0.46\pm2\cdot10^{-2}$

Table 1. Kinetic parameters assessed for MM, modified MM with product inhibition and Chrastil's model related to the hydrolysis of biomass pretreated with NaOH, HCl and Laccases.

4. Conclusions

The effect of delignification degree and biomass recovery has been quantitatively assessed through kinetic characterization of enzymatic saccharification. This procedure can be adopted for further design of hydrolysis reactors.

References

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Keywords
Enzymatic hydrolysis; Lignocellulose; Kinetic characterization