Enhancement of Cellulose Saccharification Kinetics Using an Ionic Liquid

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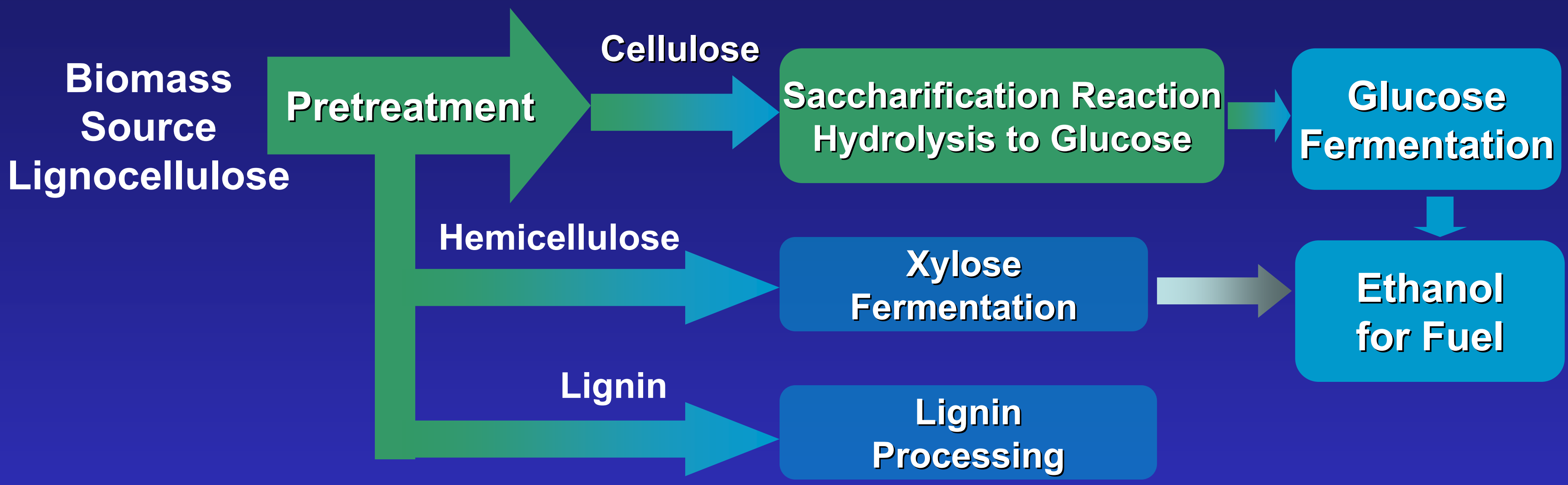
Ethanol from Biomass



Hydrolysis : critical step

- Enzymatic hydrolysis
- Acid hydrolysis

Cellulose Saccharification

Acid catalyzed hydrolysis

Advantages
- inexpensive catalyst
- modest reaction rates

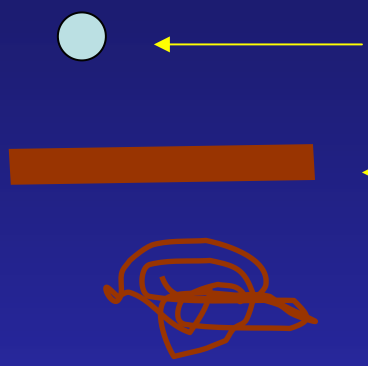
Disadvantages
- degrades glucose (inhibits fermentation)
- corrosive

Enzymatic hydrolysis using *cellulases*

Advantages
- selective for glucose
- non-corrosive

Disadvantages
- slow reaction rate
- high cost of enzyme and difficult recovery

Enzyme Hydrolysis



Enzyme molecule

Cellulose fibril

Amorphous cellulose

Slow hydrolysis

Rapid hydrolysis

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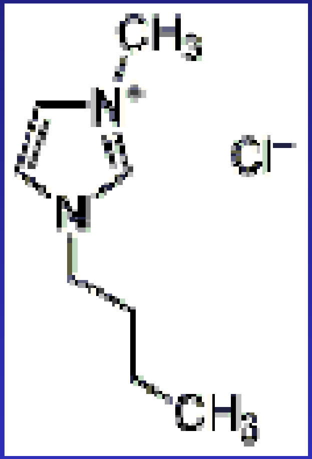
Cellulose Dissolution in Ionic Liquids

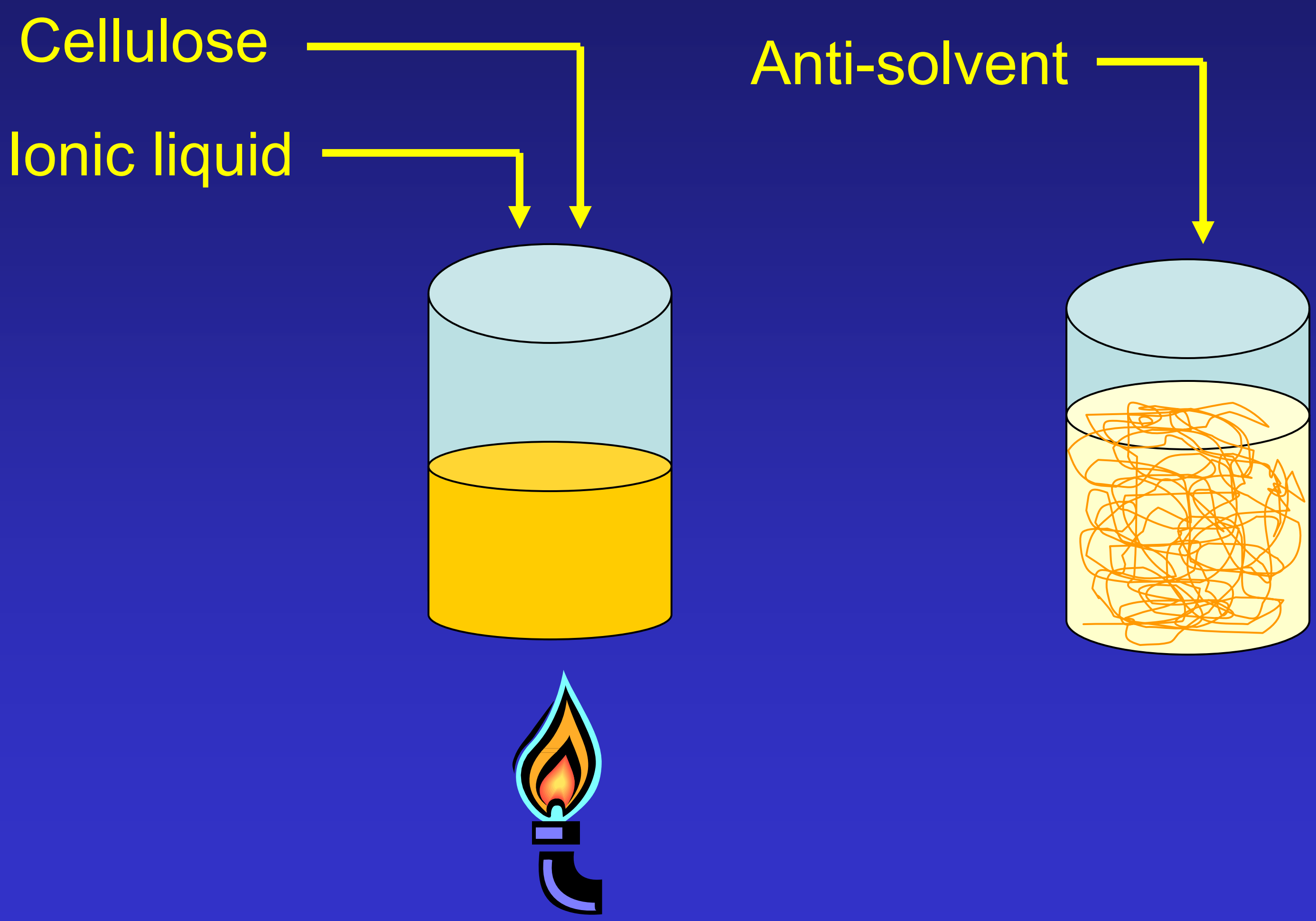


1-n-butyl-3-methyl
imidazolium chloride

- Ionic liquids (IL) are salts that melt at temperatures near ambient.
- Solvent properties can be “tuned” through the cation/anion selection
- BmimCl has been found to dissolve cellulose*

*R.P Swatloski, S.K. Spear, J.D.Holbrey, R.D. Rogers, (2002) J. Am. Chem. Soc. 124:4974-4975

Cellulose Regeneration



Cellulose Regeneration

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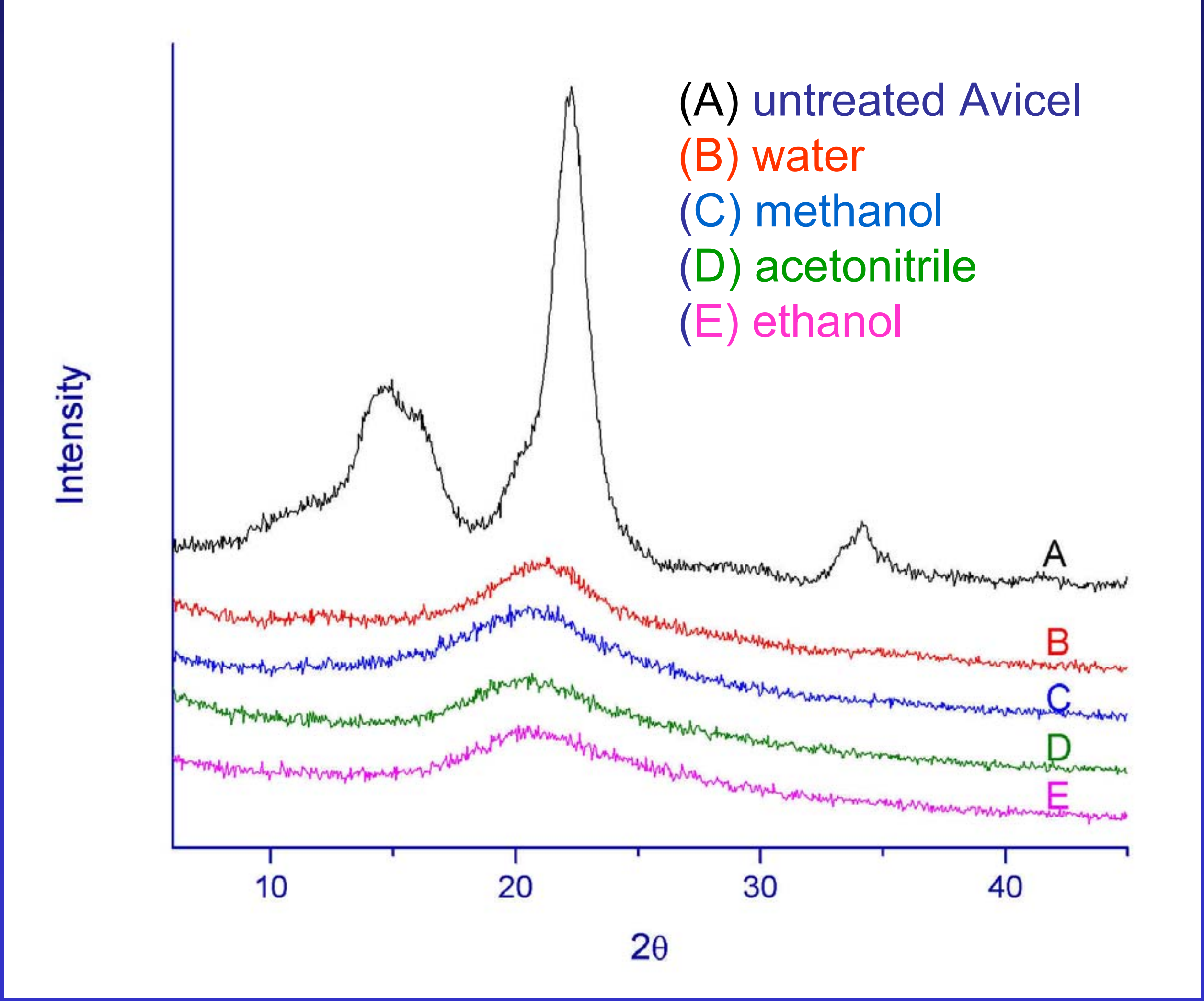
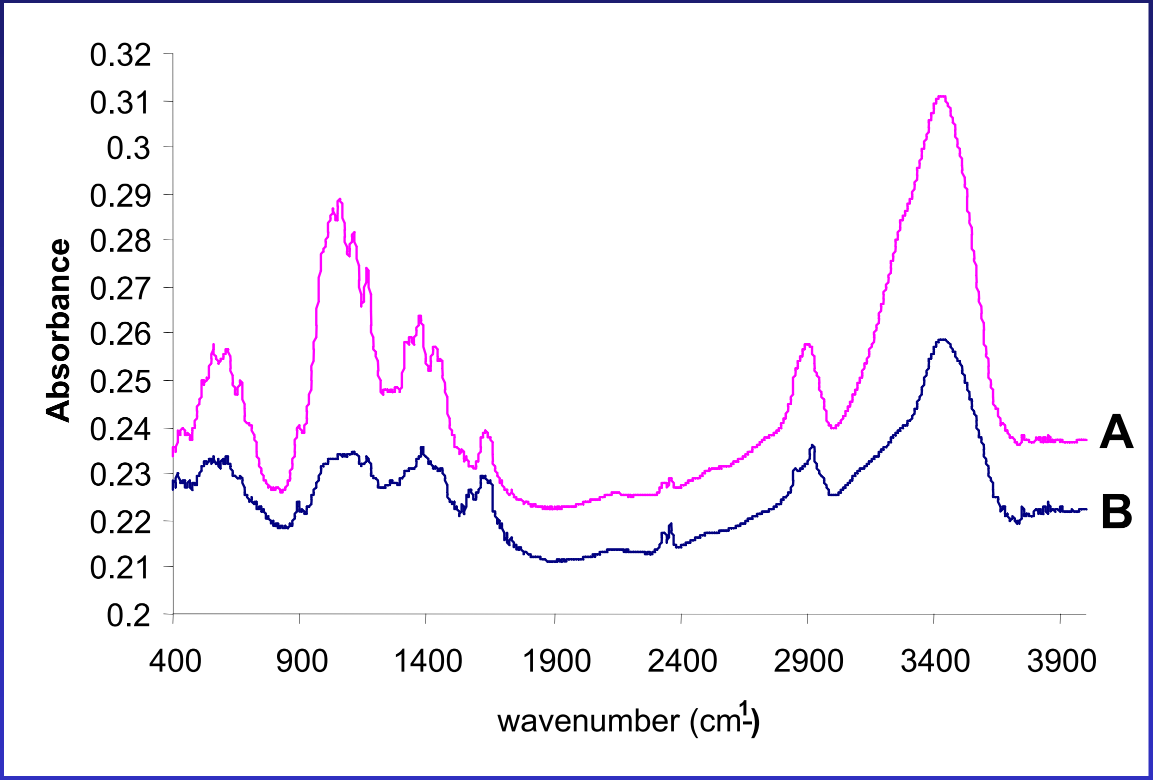
XRD of Regenerated Cellulose



FTIR of Regenerated Cellulose



A is untreated cellulose (Avicell, PH101)
B is regenerated cellulose (anti-solvent: water)

Hydrolysis Experiments

- **Enzyme hydrolysis**
  - Worthington *T. reesei* cellulase, 150 to 340 FPU/ g glucan
  - Celluclast 1.5L, 50 FPU/g glucan; with/without Novozyme 188, 0-166 CBU/g glucan
- **Substrate**
  - Untreated and IL-regenerated Avicel PH101
- **Sugar assay**
  - Total soluble reducing sugars (DNS assay)
  - Glucose formation, glucose hexokinase assay

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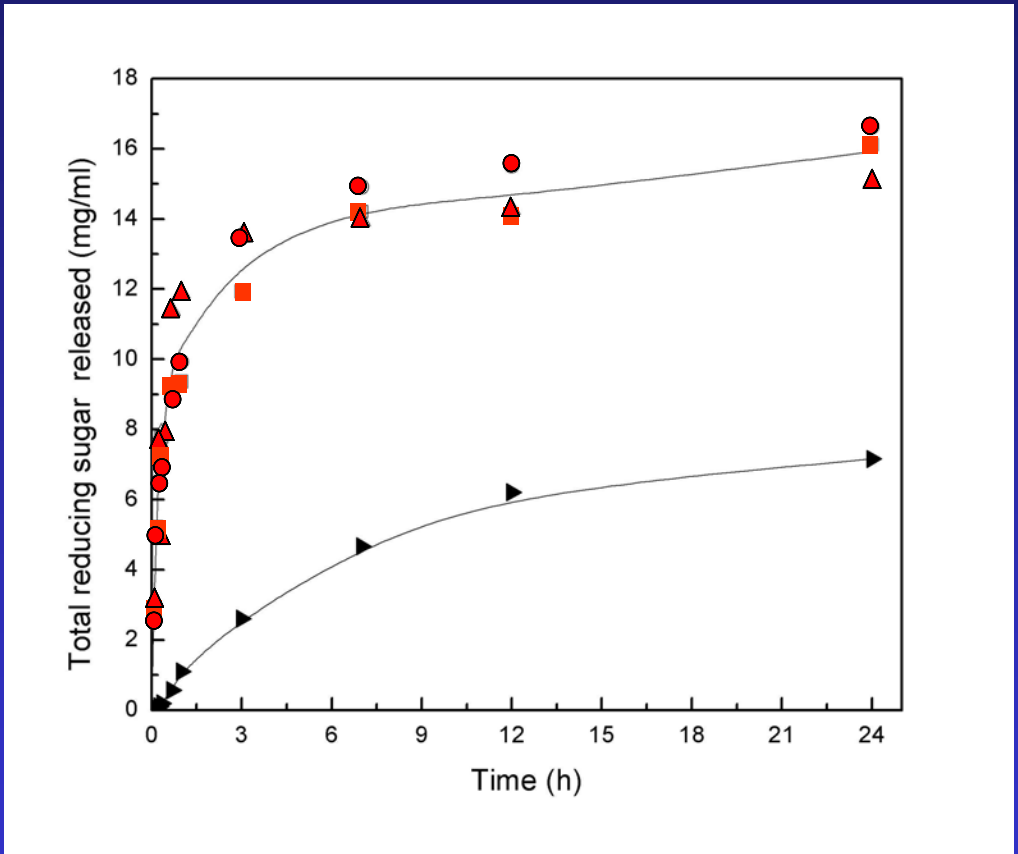
Cellulose Hydrolysis

Total soluble sugar formation



Avicel samples incubated at 130° C for 10 minutes in BmimCl.

Anti-solvent:

(■) ethanol

(●) deionized water

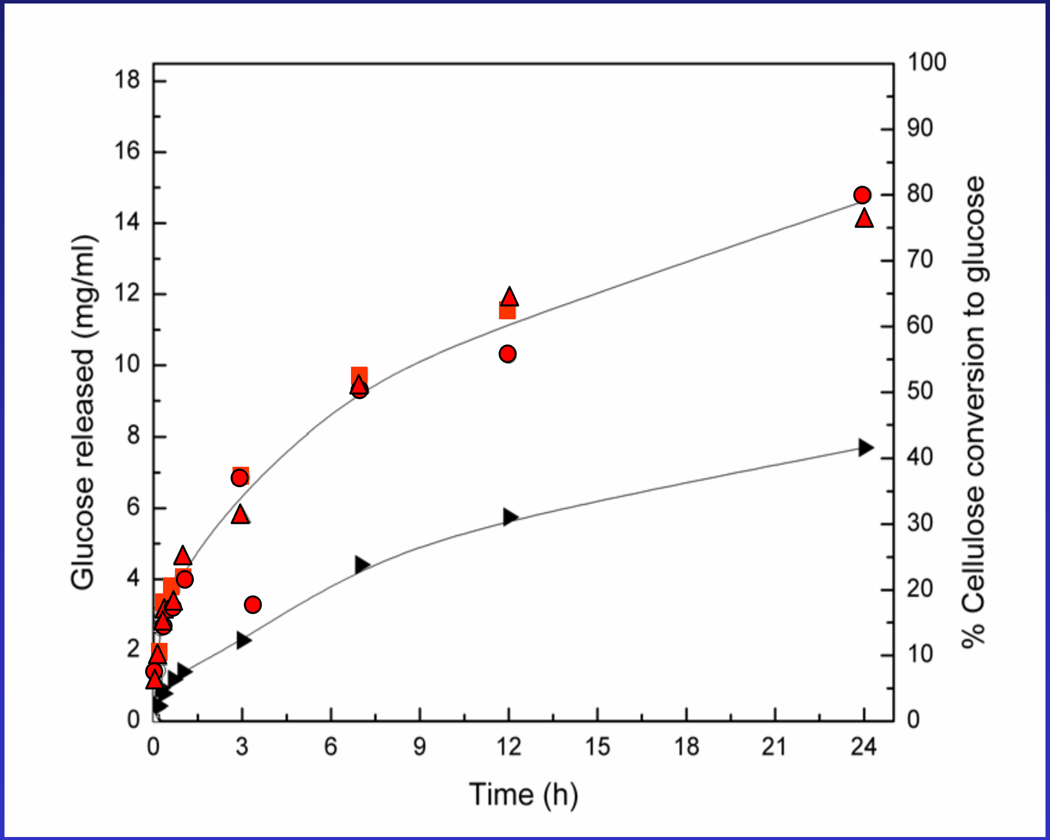
(▲) methanol

(▶) untreated Avicel

17 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 170 FPU/g glucan

Cellulose Hydrolysis

Glucose formation rate



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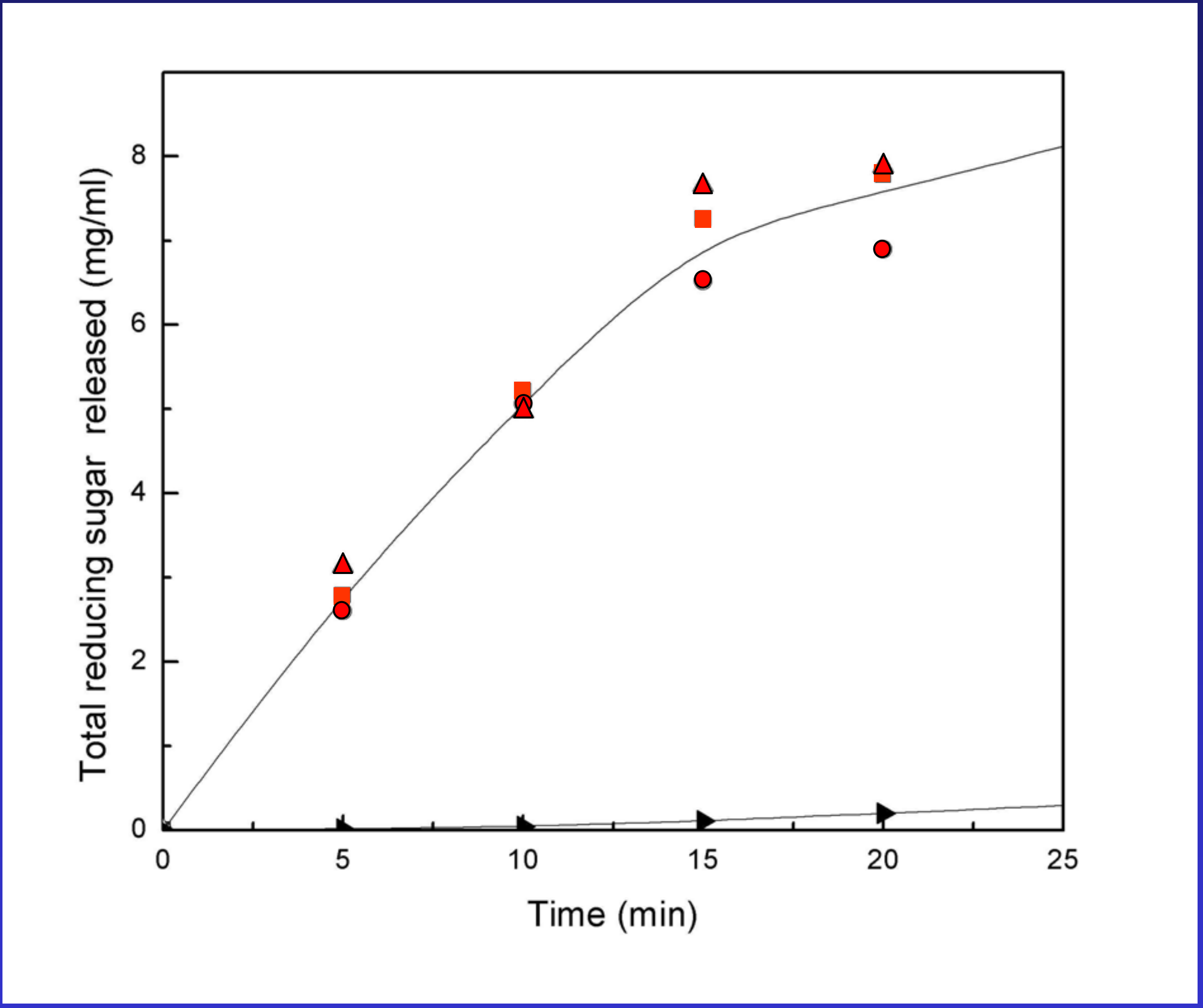
Initial Hydrolysis Rate

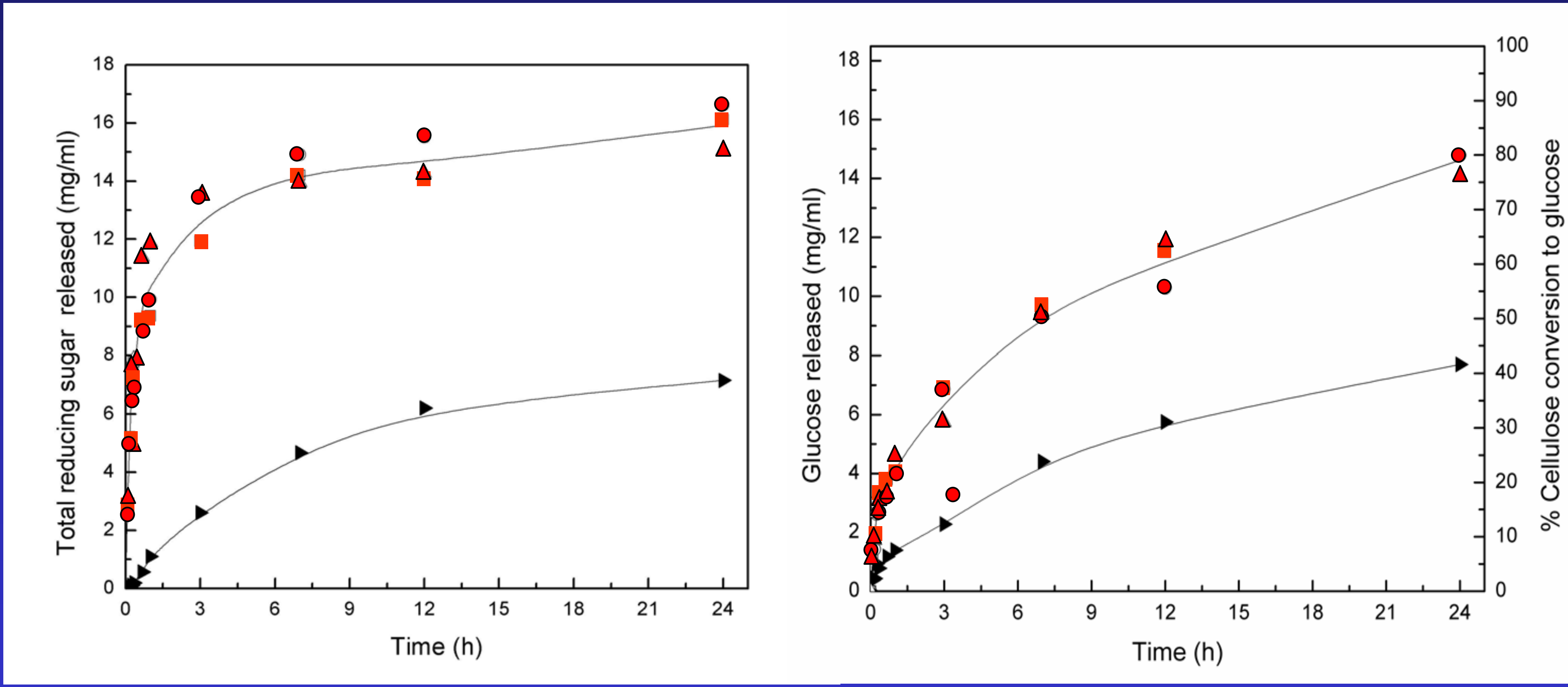


Initial Rate of Formation of Soluble sugars

Anti-solvent	Initial rate ($mg\ ml^{-1} min^{-1}$)	Rate Enhancement*
water	0.6473	52
methanol	0.6823	55
ethanol	0.6473	53
untreated	0.0125	-

*** Rate enhancement = initial rate regenerated cellulose / untreated cellulose**

Total Sugars vs. Glucose



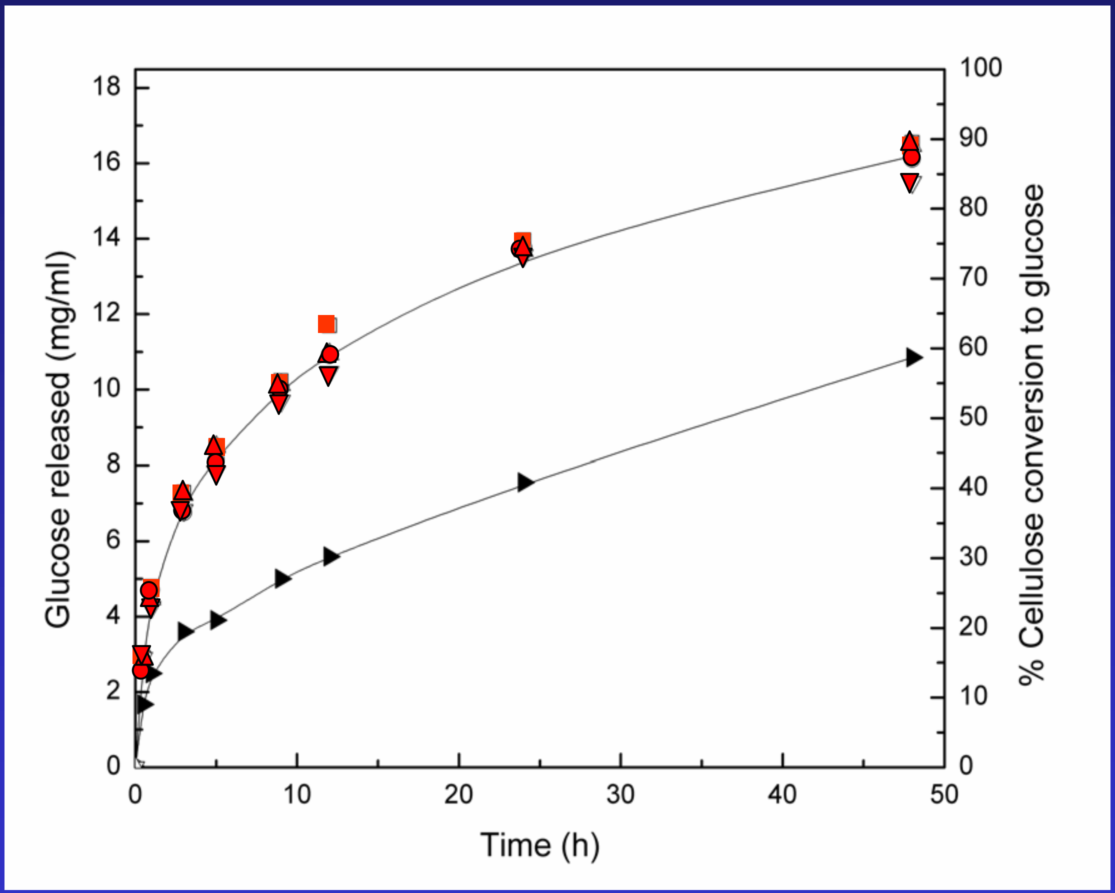
Initial Rate of Formation of Soluble Sugars

Enzyme activity		Initial Rate (mg/ml min)		Rate Enhancement
Cellulase FPU	β-glucosidase CBU	Untreated Cellulose	Regen. Cellulose	
25	0	0.0004	0.0047	12
25	83	0.0004	0.0320	71
50	0	0.0043	0.0427	10
50	83	0.0044	0.3915	89
100	0	0.0110	0.3953	36
100	83	0.0140	0.5030	36

* Rate enhancement = initial rate regenerated cellulose / untreated cellulose

Cellulose Hydrolysis

Effect of incubation time



Avicel samples incubated at 130° C for:

(■) 10 min

(●) 30 min

(▲) 1hour

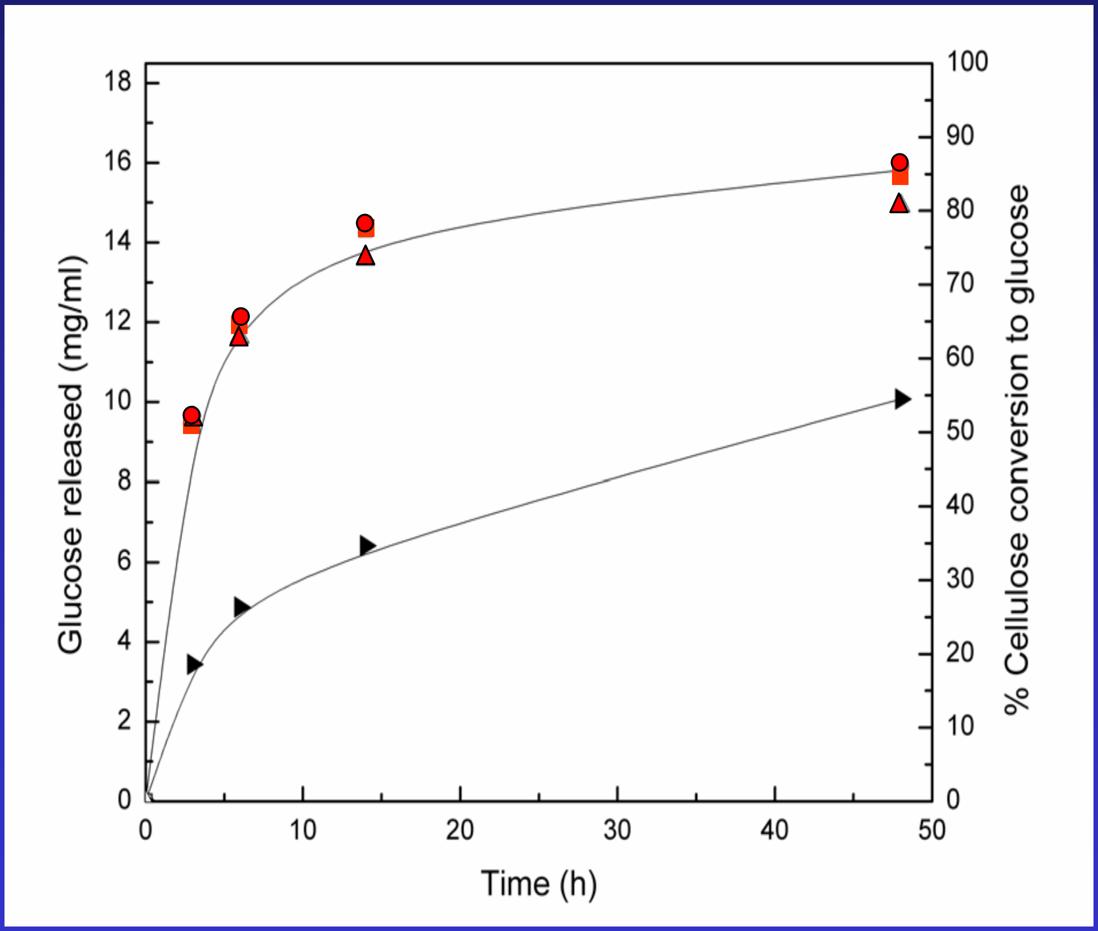
(▼) 3 hours

(▶) untreated

Precipitated with water

15 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 150 FPU/g glucan

Cellulose Hydrolysis
Effect of dissolution temperature



17 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 340 FPU/g glucan

Avicel samples incubated for 2 h at:

(■)130° C
(●) 140° C
(▲) 150° C
(▶)untreated

Precipitated with water

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Summary & Conclusions

- Simple dissolution of cellulose in the ionic liquid followed by rapid precipitation with anti-solvent is required: incubation time and temperature in IL does not affect hydrolysis.

- Regeneration appears to increase access of cellulose to endo- and exo-glucanases, resulting in significantly improved release rate of soluble sugars.

- Addition of β-glucosidase resulted in higher enzymatic hydrolysis rates.

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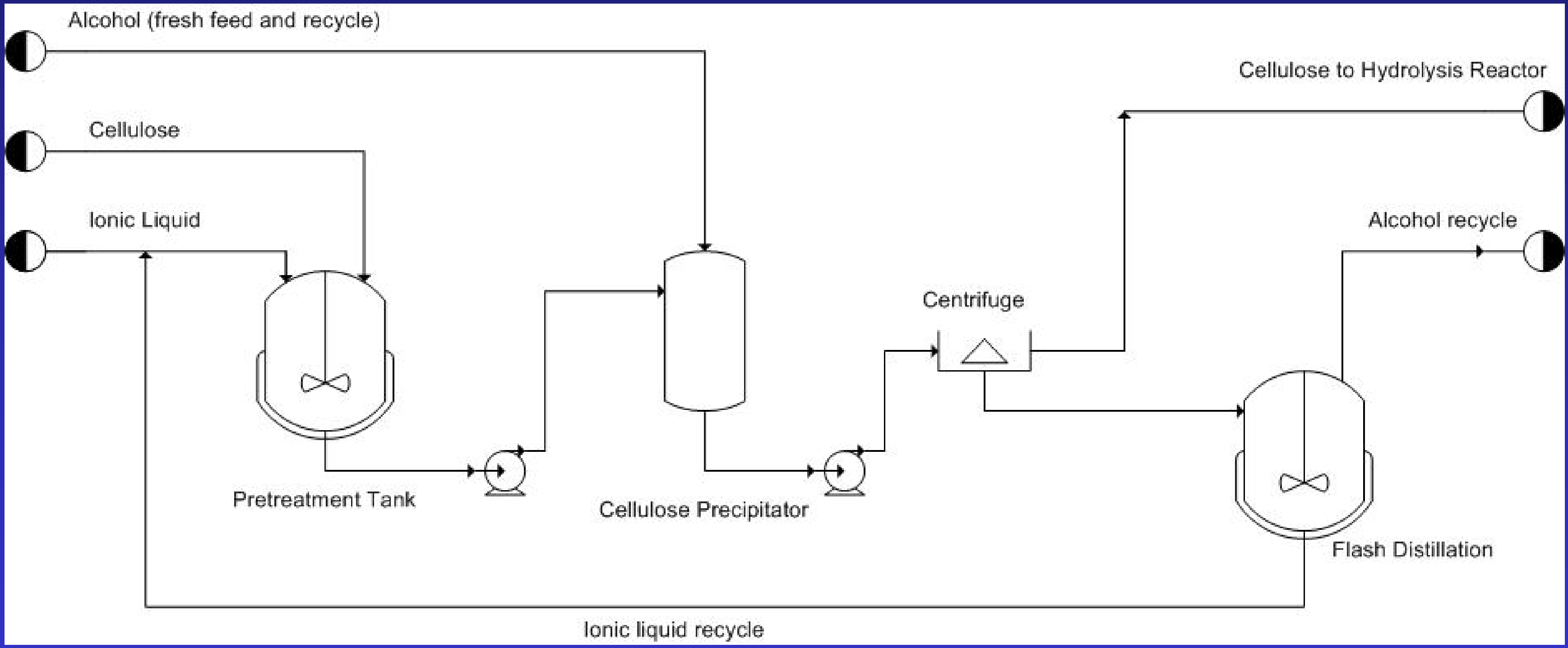
Summary & Conclusions

- Cellulose regenerated from ionic liquid solution is essentially amorphous.

- Regenerated cellulose exhibited up to a 90 fold increase in hydrolysis rates.

Process Schematic



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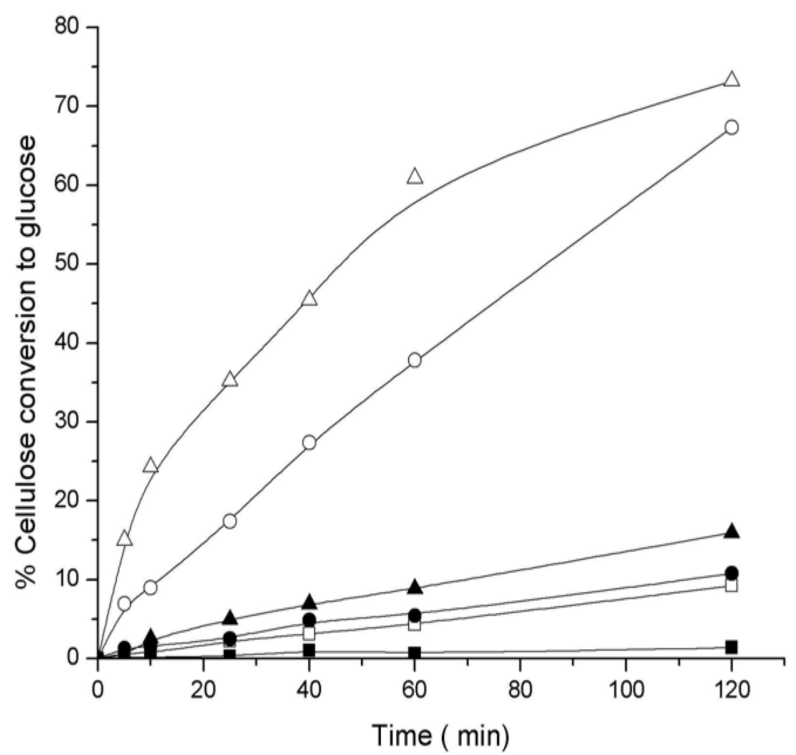
Cellulose Hydrolysis
Effect of β-glucosidase addition



15 mg/ml Avicel hydrolyzed with *T. reesei* cellulase activity of 50 FPU

Avicel samples incubated at 130 °C for 10min in BmimCl and precipitated with water

(Δ) 166 CBU/g glucan
(○) 83 CBU/g glucan
(□) no added β-glucosidase

untreated Avicel hydrolysis in closed symbols