Contents

List of Contributors xvi
Foreword xxi
Series Preface xxiii
Preface xxv
Acknowledgements xxvii

1 Introduction 1
   Charles E. Wyman
   1.1 Cellulosic Biomass: What and Why? 2
   1.2 Aqueous Processing of Cellulosic Biomass into Organic Fuels and Chemicals 3
   1.3 Attributes for Successful Pretreatment 5
   1.4 Pretreatment Options 7
   1.5 Possible Blind Spots in the Historic Pretreatment Paradigm 8
   1.6 Other Distinguishing Features of Pretreatment Technologies 9
   1.7 Book Approach 9
   1.8 Overview of Book Chapters 10
   Acknowledgements 10
   References 11

2 Cellulosic Biofuels: Importance, Recalcitrance, and Pretreatment 17
   Lee Lynd and Mark Laser
   2.1 Our Place in History 17
   2.2 The Need for Energy from Biomass 17
   2.3 The Importance of Cellulosic Biomass 18
   2.4 Potential Barriers 18
   2.5 Biological and Thermochemical Approaches to the Recalcitrance Barrier 19
   2.6 Pretreatment 20
   Acknowledgements 21
   References 21
10 Fundamentals of Biomass Pretreatment by Fractionation

Poulomi Sannigrahi and Arthur J. Ragauskas

10.1 Introduction

10.2 Organosolv Pretreatment
   10.2.1 Organosolv Pulping
   10.2.2 Overview of Organosolv Pretreatment
   10.2.3 Solvents and Catalysts for Organosolv Pretreatment
   10.2.4 Fractionation of Biomass during Organosolv Pretreatment

10.3 Nature of Organosolv Lignin and Chemistry of Organosolv Delignification
   10.3.1 Composition and Structure of Organosolv Lignin
   10.3.2 Mechanisms of Organosolv Delignification
   10.3.3 Commercial Applications of Organosolv Lignin

10.4 Structural and Compositional Characteristics of Cellulose

10.5 Co-products of Biomass Fractionation by Organosolv Pretreatment
   10.5.1 Hemicellulose
   10.5.2 Furfural
   10.5.3 Hydroxymethylfurfural (HMF)
   10.5.4 Levulinic Acid
   10.5.5 Acetic Acid

10.6 Conclusions and Recommendations

Acknowledgements
References
12 Comparative Performance of Leading Pretreatment Technologies for Biological Conversion of Corn Stover, Poplar Wood, and Switchgrass to Sugars


12.1 Introduction
12.2 Materials and Methods
  12.2.1 Feedstocks
  12.2.2 Enzymes
  12.2.3 CAFI Pretreatments
  12.2.4 Material Balances
  12.2.5 Free Sugars and Extraction
12.3 Yields of Xylose and Glucose from Pretreatment and Enzymatic Hydrolysis
  12.3.1 Yields from Corn Stover
  12.3.2 Yields from Standard Poplar
  12.3.3 Yields from Dacotah Switchgrass
12.4 Impact of Changes in Biomass Sources
12.5 Compositions of Solids Following CAFI Pretreatments
  12.5.1 Composition of Pretreated Corn Stover Solids
  12.5.2 Composition of Pretreated Switchgrass Solids
  12.5.3 Composition of Pretreated Poplar Solids
  12.5.4 Overall Trends in Composition of Pretreated Biomass Solids and Impact on Enzymatic Hydrolysis
12.6 Pretreatment Conditions to Maximize Total Glucose Plus Xylose Yields
12.7 Implications of the CAFI Results
12.8 Closing Thoughts
Acknowledgements
References

13 Effects of Enzyme Formulation and Loadings on Conversion of Biomass Pretreated by Leading Technologies

Rajesh Gupta and Y.Y. Lee

13.1 Introduction
13.2 Synergism among Cellulolytic Enzymes
13.3 Hemicellulose Structure and Hemicellulolytic Enzymes
13.4 Substrate Characteristics and Enzymatic Hydrolysis
13.5 Xylanase Supplementation for Different Pretreated Biomass and Effect of β-Xylosidase
13.6 Effect of β-Glucosidase Supplementation
13.7 Effect of Pectinase Addition
13.8 Effect of Feruloyl Esterase and Acetyl Xylan Esterase Addition
13.9 Effect of α-L-arabinofuranosidase and Mannanase Addition
13.10 Use of Lignin-degrading Enzymes (LDE)
13.11 Effect of Inactive Components on Biomass Hydrolysis
13.12 Adsorption and Accessibility of Enzyme with Different Cellulosic Substrates
13.13 Tuning Enzyme Formulations to the Feedstock 272
13.14 Summary 273
References 274

14 Physical and Chemical Features of Pretreated Biomass that Influence Macro-/Micro-accessibility and Biological Processing 281
Rajeev Kumar and Charles E. Wyman

14.1 Introduction 281
14.2 Definitions of Macro-/Micro-accessibility and Effectiveness 283
14.3 Features Influencing Macro-accessibility and their Impacts on Enzyme Effectiveness 284
14.3.1 Lignin 284
14.3.2 Hemicellulose 286
14.4 Features Influencing Micro-accessibility and their Impact on Enzymes Effectiveness 289
14.4.1 Cellulose Crystallinity (Structure) 289
14.4.2 Cellulose Chain Length/Reducing Ends 291
14.5 Concluding Remarks 293
Acknowledgements 296
References 296

15 Economics of Pretreatment for Biological Processing 311
Ling Tao, Andy Aden and Richard T. Elander

15.1 Introduction 311
15.2 Importance of Pretreatment 311
15.3 History of Pretreatment Economic Analysis 313
15.4 Methodologies for Economic Assessment 314
15.5 Overview of Pretreatment Technologies 315
15.5.1 Acidic Pretreatments 315
15.5.2 Alkaline Pretreatments 315
15.5.3 Solvent-based Pretreatments 316
15.6 Comparative Pretreatment Economics 316
15.6.1 Modeling Basis and Assumptions for Comparative CAFI Analysis 317
15.6.2 CAFI Project Comparative Data 320
15.6.3 Reactor Design and Costing Data 320
15.6.4 Comparison of Sugar and Ethanol Yields 324
15.6.5 Comparison of Pretreatment Capital Costs 325
15.6.6 Comparison of MESP 326
15.7 Impact of Key Variables on Pretreatment Economics 327
15.7.1 Yield 327
15.7.2 Conversion to Oligomers/Monomers (Shift of Burden between Enzymes and Pretreatment) 328
15.7.3 Biomass Loading/Concentration 328
15.7.4 Chemical Loading/Recovery/Metallurgy 329
15.7.5 Reaction Conditions: Pressure, Temperature, Residence Time 330
15.7.6 Reactor Orientation: Horizontal/Vertical 330
15.7.7 Batch versus Continuous Processing 330
15.8 Future Needs for Evaluation of Pretreatment Economics 331
15.9 Conclusions 332
Acknowledgements 332
References 332

16 Progress in the Summative Analysis of Biomass Feedstocks for Biofuels Production 335
F.A. Agblevor and J. Pereira

16.1 Introduction 335
16.2 Preparation of Biomass Feedstocks for Analysis 337
16.3 Determination of Non-structural Components of Biomass Feedstocks 338
  16.3.1 Moisture Content of Biomass Feedstocks 338
  16.3.2 Determination of Ash in Biomass 338
  16.3.3 Protein Content of Biomass 338
  16.3.4 Extractives Content of Biomass 339
16.4 Quantitative Determination of Lignin Content of Biomass 340
16.5 Quantitative Analysis of Sugars in Lignocellulosic Biomass 342
  16.5.1 Holocellulose Content of Plant Cell Walls 342
  16.5.2 Monoethanolamine Method for Cellulose Determination 343
16.6 Chemical Hydrolysis of Biomass Polysaccharides 343
  16.6.1 Mineral Acid Hydrolysis 343
  16.6.2 Trifluoroacetic Acid (TFA) 344
  16.6.3 Methanalysis 344
16.7 Analysis of Monosaccharides 345
  16.7.1 Colorimetric Analysis of Biomass Monosaccharides 345
  16.7.2 Gas Chromatographic Sugar Analysis 345
16.8 Gas Chromatography-Mass Spectrometry (GC/MS) 347
16.9 High-performance Liquid Chromatographic Sugar Analysis 347
16.10 NMR Analysis of Biomass Sugars 349
16.11 Conclusions 349
References 349

17 High-throughput NIR Analysis of Biomass Pretreatment Streams 355
Bonnie R. Hames

17.1 Introduction 355
17.2 Rapid Analysis Essentials 356
  17.2.1 Rapid Spectroscopic Techniques 357
  17.2.2 Calibration and Validation Samples 358
  17.2.3 Quality Calibration Data for Each Calibration Sample 359
  17.2.4 Multivariate Analysis to Resolve Complex Sample Spectra 362
  17.2.5 Validation of New Methods 364
  17.2.6 Standard Reference Materials and Protocols for Ongoing QA/QC 364
17.3 Summary 366
References 367
18  Plant Biomass Characterization: Application of Solution- and Solid-state
NMR Spectroscopy
Yunjiao Pu, Bassem Hallac and Arthur J. Ragauskas

18.1 Introduction 369
18.2 Plant Biomass Constituents 370
18.3 Solution-state NMR Characterization of Lignin 371
  18.3.1 Lignin Sample Preparation 372
  18.3.2 \(^1\text{H}\) NMR Spectroscopy 372
  18.3.3 \(^{13}\text{C}\) NMR Spectroscopy 372
  18.3.4 HSQC Correlation Spectroscopy 375
  18.3.5 \(^{31}\text{P}\) NMR Spectroscopy 377
18.4 Solid-state NMR Characterization of Plant Cellulose 381
  18.4.1 CP/MAS \(^{13}\text{C}\) NMR Analysis of Cellulose 381
  18.4.2 Cellulose Crystallinity 383
  18.4.3 Cellulose Ultrastructure 385
18.5 Future Perspectives 387
Acknowledgements 387
References 387

19  Xylooligosaccharides Production, Quantification, and Characterization in Context
of Lignocellulosic Biomass Pretreatment
Qing Qing, Hongjia Li, Rajeev Kumar and Charles E. Wyman

19.1 Introduction 391
  19.1.1 Definition of Oligosaccharides 391
  19.1.2 Types of Oligosaccharides Released during Lignocellulosic
      Biomass Pretreatment 392
  19.1.3 The Importance of Measuring Xylooligosaccharides 392
19.2 Xylooligosaccharides Production 394
  19.2.1 Thermochemical Production of XOs 394
  19.2.2 Production of XOs by Enzymatic Hydrolysis 396
19.3 Xylooligosaccharides Separation and Purification 397
  19.3.1 Solvent Extraction 397
  19.3.2 Adsorption by Surface Active Materials 397
  19.3.3 Chromatographic Separation Techniques 398
  19.3.4 Membrane Separation 399
  19.3.5 Centrifugal Partition Chromatography 401
19.4 Characterization and Quantification of Xylooligosaccharides 402
  19.4.1 Measuring Xylooligosaccharides by Quantification of
      Reducing Ends 402
  19.4.2 Characterizing Xylooligosaccharides Composition 402
  19.4.3 Direct Characterization of Different DP Xylooligosaccharides
  19.4.4 Determining Detailed Structures of Oligosaccharides
      by MS and NMR 408
19.5 Concluding Remarks 408
Acknowledgements 409
References 410
20 Experimental Pretreatment Systems from Laboratory to Pilot Scale
Richard T. Elander

20.1 Introduction 417
20.2 Laboratory-scale Pretreatment Equipment 421
  20.2.1 Heating and Cooling Capability 421
  20.2.2 Contacting of Biomass Particles with Water and/or Pretreatment
      Chemicals 421
  20.2.3 Mass and Heat Transfer 422
  20.2.4 Proper Materials of Construction 423
  20.2.5 Instrumentation and Control Systems 424
  20.2.6 Translating to Pilot-scale Pretreatment Systems 424
20.3 Pilot-scale Batch Pretreatment Equipment 424
20.4 Pilot-scale Continuous Pretreatment Equipment 427
  20.4.1 Feedstock Handling and Size Reduction 427
  20.4.2 Pretreatment Chemical and Water Addition 429
  20.4.3 Pressurized Continuous Pretreatment Feeder Equipment 432
  20.4.4 Pretreatment Reactor Throughput and Residence Time Control 436
  20.4.5 Reactor Discharge Devices 438
  20.4.6 Blow-down Vessel and Flash Vapor Recovery 438
20.5 Continuous Pilot-scale Pretreatment Reactor Systems 439
  20.5.1 Historical Development of Pilot-scale Reactor Systems 439
  20.5.2 NREL Gravity-flow Reactor Systems 441
20.6 Summary 445
Acknowledgements 446
References 447

21 Experimental Enzymatic Hydrolysis Systems
Todd Lloyd and Chaogang Liu

21.1 Introduction 451
21.2 Cellulases
  21.2.1 Endoglucanase 452
  21.2.2 Celllobiohydrolase 453
  21.2.3 β-glucosidase 453
21.3 Hemicellulases 453
21.4 Kinetics of Enzymatic Hydrolysis
  21.4.1 Empirical Models 454
  21.4.2 Michaelis–Menten-based Models 455
  21.4.3 Adsorption in Cellulose Hydrolysis Models 456
  21.4.4 Rate Limitations and Decreasing Rates with Increasing
      Conversion 457
  21.4.5 Summary of Enzyme Reaction Kinetics 459
21.5 Experimental Hydrolysis Systems 460
  21.5.1 Laboratory Protocols 460
  21.5.2 Considerations for Scale-up of Hydrolysis Processes 463
21.6 Conclusion 465
References 465
## 22 High-throughput Pretreatment and Hydrolysis Systems for Screening Biomass Species in Aqueous Pretreatment of Plant Biomass

*Jaclyn DeMartini and Charles E. Wyman*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.1 Introduction: The Need for High-throughput Technologies</td>
<td>471</td>
</tr>
<tr>
<td>22.2 Previous High-throughput Systems and Application to Pretreatment and Enzymatic Hydrolysis</td>
<td>472</td>
</tr>
<tr>
<td>22.3 Current HTPH Systems</td>
<td>473</td>
</tr>
<tr>
<td>22.4 Key Steps in HTPH Systems</td>
<td>478</td>
</tr>
<tr>
<td>22.4.1 Material Preparation</td>
<td>478</td>
</tr>
<tr>
<td>22.4.2 Material Distribution</td>
<td>479</td>
</tr>
<tr>
<td>22.4.3 Pretreatment and Enzymatic Hydrolysis</td>
<td>480</td>
</tr>
<tr>
<td>22.4.4 Sample Analysis</td>
<td>481</td>
</tr>
<tr>
<td>22.5 HTPH Philosophy, Difficulties, and Limitations</td>
<td>482</td>
</tr>
<tr>
<td>22.6 Examples of Research Enabled by HTPH Systems</td>
<td>484</td>
</tr>
<tr>
<td>22.7 Future Applications</td>
<td>485</td>
</tr>
<tr>
<td>22.8 Conclusions and Recommendations</td>
<td>485</td>
</tr>
<tr>
<td>References</td>
<td>486</td>
</tr>
</tbody>
</table>

## 23 Laboratory Pretreatment Systems to Understand Biomass Deconstruction

*Bin Yang and Melvin Tucker*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.1 Introduction</td>
<td>489</td>
</tr>
<tr>
<td>23.2 Laboratory-scale Batch Reactors</td>
<td>491</td>
</tr>
<tr>
<td>23.2.1 Sealed Glass Reactors</td>
<td>491</td>
</tr>
<tr>
<td>23.2.2 Tubular Reactors</td>
<td>492</td>
</tr>
<tr>
<td>23.2.3 Mixed Reactors</td>
<td>495</td>
</tr>
<tr>
<td>23.2.4 Zipperclave</td>
<td>496</td>
</tr>
<tr>
<td>23.2.5 Microwave Reactors</td>
<td>497</td>
</tr>
<tr>
<td>23.2.6 Steam Reactors</td>
<td>499</td>
</tr>
<tr>
<td>23.3 Laboratory-scale Continuous Pretreatment Reactors</td>
<td>501</td>
</tr>
<tr>
<td>23.4 Deconstruction of Biomass with Bench-Scale Pretreatment Systems</td>
<td>503</td>
</tr>
<tr>
<td>23.5 Heat and Mass Transfer</td>
<td>505</td>
</tr>
<tr>
<td>23.5.1 Mass Transfer</td>
<td>506</td>
</tr>
<tr>
<td>23.5.2 Direct and Indirect Heating</td>
<td>506</td>
</tr>
<tr>
<td>23.6 Biomass Handling and Comminuting</td>
<td>508</td>
</tr>
<tr>
<td>23.7 Construction Materials</td>
<td>508</td>
</tr>
<tr>
<td>23.7.1 Overall Considerations</td>
<td>508</td>
</tr>
<tr>
<td>23.7.2 Materials of Construction</td>
<td>509</td>
</tr>
<tr>
<td>23.8 Criteria of Reactor Selection and Applications</td>
<td>510</td>
</tr>
<tr>
<td>23.8.1 Effect of High/Low Solids Concentration on Reactor Choices</td>
<td>510</td>
</tr>
<tr>
<td>23.8.2 Role of Heat-up and Cool-down Rates in Laboratory Reactor Selection</td>
<td>510</td>
</tr>
<tr>
<td>23.8.3 Effect of Mixing and Catalyst Impregnation on Reactor Design</td>
<td>510</td>
</tr>
<tr>
<td>23.8.4 High Temperatures and Short Residence Times Result in High Yields</td>
<td>511</td>
</tr>
</tbody>
</table>