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1 Enzymatic hydrolysis of biomass at high-solids loadings – A review

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13 **Abstract**

14 Enzymatic hydrolysis is the unit operation in the lignocellulose conversion process that
15 utilizes enzymes to depolymerize lignocellulosic biomass. The saccharide components released
16 are the feedstock for fermentation. When performed at high-solids loadings ($\geq 15\%$ solids, w/w),
17 enzymatic hydrolysis potentially offers many advantages over conversions performed at low- or
18 moderate-solids loadings, including increased sugar and ethanol concentrations and decreased
19 capital and operating costs.

20 The goal of this review is to provide a consolidated source of information on studies
21 using high-solids loadings in enzymatic hydrolysis. Included in this review is a brief discussion
22 of the limitations, such as a lack of available water, difficulty with mixing and handling,
23 insufficient mass and heat transfer, and increased concentration of inhibitors, associated with the
24 use of high solids, as well as descriptions and findings of studies that performed enzymatic
25 hydrolysis at high-solids loadings. Reactors designed and/or equipped for improved handling of
26 high-solids slurries are also discussed. Lastly, this review includes a brief discussion of some of
27 the operations that have successfully scaled-up and implemented high-solids enzymatic
28 hydrolysis at pilot- and demonstration-scale facilities.

29

30 Keywords: High-solids loadings; enzymatic hydrolysis; lignocellulose conversion; reactor
31 design; corn stover; straw; woody biomass

32 **1. Introduction**

33 Lignocellulose is the largest renewable source of carbon on the planet, as it is the main
34 structural component of plants. Energy from lignocellulosic biomass has been tapped as one
35 possible solution to decrease the United States' foreign dependence on petroleum, as well as
36 serve as a more environmentally friendly source of energy. Lignocellulose can either be
37 processed thermochemically or biochemically, depending on the desired product. The
38 biorefinery concept is thought to be the desired model for biomass processing, where all of the
39 biomass is exploited. The suite of products would be dictated by the market and selected to
40 extract the greatest value possible out of lignocellulose (Figure 1).

41 Enzymatic hydrolysis of lignocellulose has long been studied as a method to
42 depolymerize the biomass into fermentable sugars for conversion to biofuels and biochemicals,
43 with a more recent focus on operating at high-solids loadings. It has been suggested that
44 enzymatic hydrolysis conducted at high-solids loadings will be necessary to render the
45 lignocellulosic conversion process more economically feasible. A process is considered "high
46 solids" if the ratio of solids/liquid is such that very little to no free water is present in the slurry
47 [1] or roughly a solids loadings $\geq 15\%$ (w/w).

48 Enzymatic hydrolysis performed at high-solids loadings offers several advantages over
49 low- and moderate-solids loadings, the main one being final sugar concentrations are higher [2,
50 3]. In theory, higher sugar concentrations translate into higher ethanol concentrations, which
51 could reduce energy use and costs associated with the distillation process [4, 5]. For the purpose
52 of this paper, the term "concentration" refers to the amount of a component dissolved in a given
53 volume of liquid, while the terms "yield" and "conversion" refer to the quantity of a product
54 obtained expressed as a percentage of the theoretical maximum. Distillation is most economical

55 when the ethanol concentration is $\geq 4\%$ (w/w). In order to obtain this ethanol yield, glucose
56 yields must be at least 8% (w/w), which translated into a lignocellulose loading of $\geq 20\%$ (w/w)
57 for enzymatic hydrolysis [6]. These estimates only account for conversion of cellulose;
58 however, as improvements are made to hemicellulose conversion (hydrolysis and fermentation)
59 technologies that work in combination with cellulose conversion, this initial solids loadings
60 estimate may decrease. Another potential advantage is the reduction of capital and production
61 costs. Smaller equipment and/or fewer reactors can be utilized to produce an equivalent output
62 [7, 8]. Fewer reactors also translate into reduced energy demands for heating, cooling and
63 mixing [3, 5], although the latter aspect may be a point of contention as increased solids makes
64 effective mixing more difficult. Additionally, less water is needed, which reduces the cost of
65 disposal or treatment of process water.

66 The goal for this review is to provide a consolidated source of information for the latest
67 technological advances for managing enzymatic hydrolysis at high-solids loadings. Following a
68 brief discussion of the factors limiting enzymatic hydrolysis at high solids, various aspects and
69 approaches pertaining to hydrolysis operating conditions are detailed. Additionally, reactors
70 designed to overcome some of the limitations associated with high-solids hydrolysis, as well as
71 pilot- and demonstration-scale plants operating at high-solids loadings are discussed. Lastly, the
72 authors comment on the envisioned direction for high-solids hydrolysis research, as well as the
73 necessary advances this technology must make to become commercially viable.

74

75 **2. Factors Limiting High-Solids Enzymatic Hydrolysis**

76 As solids loading increases, challenges that were negligible in low-solid systems become
77 more prominent, which has also been noted in high solids pretreatment [9]. One of the major

78 challenges for enzymatic hydrolysis at high solids loading is the lack of available water in the
79 reactor. Water is essential to effective hydrolysis for two reasons: mass transfer and lubricity.
80 Water increases the effectiveness of the enzymatic and chemical reactions, mainly by providing a
81 medium for solubilizing and aiding in the mass transfer of products. Water also reduces the
82 viscosity of the slurry by increasing the lubricity of the particles, which decreases the required
83 shear stress necessary to produce a given shear rate, allowing lower power input for mixing [1,
84 10]. The physical and chemical properties of the specific biomass affect the way biomass
85 absorbs water. As solids loadings approach 20% (w/w), the liquid fraction becomes fully
86 absorbed into the biomass leaving little free water [1]. With lower amounts of free water, the
87 apparent viscosity of the mixture increases, and consequently mixing and handling of material
88 become more difficult.

89 Gervais, Benoussan and Grajek [11] investigated the relationship between water content
90 and water activity on microorganisms in a high-solids cellulose environment. No free water
91 occurs when the matric potential of the substrate holds the water more tightly within its pores
92 than the gravitational force acts on it. The water potential (= osmotic potential + matric
93 potential) of the system is such that content affects mass transfer by limiting diffusion of
94 products away from enzyme [11]. Not only can the enzymes release compounds from the
95 biomass that are inhibitory to the organisms used in the fermentation step, but the sugar products
96 they produce are known inhibitors in the enzymatic feedback mechanism [2, 12, 13]. For
97 example, cellobiose inhibits the cellulase. Typically, cellulase is supplemented with β -
98 glucosidase to reduce the inhibition by cellobiose. However, it has recently been shown that
99 hydrolysis rates of cellulase and β -glucosidase are greatly impacted by hemicellulose-derived
100 products, like xylose, xylan and xylo-oligomers [14-16]. Pretreatment methods that do not

101 remove these products or enzyme cocktails that include xylanases may have detrimental effects
102 on glucose yields. While inhibition occurs at low solids, as well as at high solids, the increased
103 concentration of inhibitors, in addition to the reduced mass transfer rate away from the enzyme,
104 makes inhibition more apparent at high-solids loadings.

105 The challenges apparent at high solids are interrelated, so a less-than-ideal condition in
106 one property exacerbates the negative effects of another property. For example, the substrates'
107 physio/chemical properties affect the water retention value (WRV) of the biomass. A high WRV
108 (due to high-solids content and the specific properties of the substrate) reduces the diffusion of
109 inhibitors away from the enzymatic reaction, and increases the apparent viscosity of the mixture,
110 thereby increasing the difficulty of stirring the mixture to assist with diffusion. Zhang et al. [17]
111 found that the energy required to mix increased one order of magnitude when they increased the
112 solids loading of pretreated corn stover from 15% to 30% w/w (79.5 MJ/t slurry to 1009.2 MJ/t
113 slurry, respectively) to produce 854.9 and 1723.2 MJ/t slurry of ethanol respectively. The higher
114 solids loading did indeed achieve the goal of producing a higher concentration of ethanol in the
115 broth; however, over half of the energy produced in the ethanol was consumed in the mixing to
116 achieve the higher concentration of ethanol (compared to 9% of the energy needed to mix the
117 system producing the lower concentration of ethanol.

118 While it is widely recognized that increasing the solids content in a conversion process
119 increases product concentration [18], it is also widely recognized that the increase in yield is not
120 linear with increasing initial solids content because yield (percent conversion) decreases with
121 initial solids content (slope is a function of substrate type, pretreatment, and enzyme loading,
122 among other things) [10]. In fact, this well-recognized challenge was observed so often that
123 Kristensen et al. [10] coined the term *solids effect* to describe the persistence of a measured

124 reduction in conversion when solids loadings are increased. The scientific community has yet to
125 come to agreement as to the cause of the solids effect; however, theories include substrate
126 effects, product inhibition, water content and enzyme adsorption characteristics, just to name a
127 few [10].

128 Other challenges specific to high-solids enzymatic hydrolysis include long hydrolysis
129 times. Enzymatic hydrolysis is typically thought to be the bottleneck of the entire conversion
130 process in terms of both time and money, since the reaction time needed for most enzymes to
131 convert lignocellulose into sufficient glucose concentrations for fermentation is on the order of
132 days (usually ≥ 3 days). Long hydrolysis times can only be reduced so much by increasing
133 enzyme loading. Recent studies have suggested that enzymes can overcrowd accessible
134 cellulose sites, thus not reaching the full hydrolytic potential for the given enzyme loading [19,
135 20]. Adjacent cellulose chains are $\sim 4\text{-}6$ Å apart, whereas the diameter of the cellulases is about
136 10-fold larger at about 45 Å (Figure 2). Furthermore, as in low-solids hydrolysis, the cost of the
137 enzyme is also a limiting factor. Enzyme is typically added on a per weight of substrate basis.
138 As the solids loading increases so must the amount of enzyme. While the cost of enzymes has
139 decreased drastically over the years due to intense research developing cheaper production
140 schemes, the cost is still at a level that makes this step in the conversion process one of the most
141 expensive. Finding or developing enzymes with a high activity and inexpensive method of
142 production would greatly benefit the entire conversion process. Moreover, it is also important to
143 evaluate the economics when determining the balance between the loadings applied to the
144 lignocellulose and the amount of time needed to reach sufficient glucose concentrations.

145

146 3. Impacting Rheology of High-Solids Mixtures

147 Rheology is the branch of physics that deals with the deformation and flow of matter. At
148 higher lignocellulose loadings, fundamental understanding of the rheology of these suspensions
149 becomes a powerful tool in designing conversion equipment and processes [21-24]. Factors
150 which contribute to the rheological properties of a suspension include particle size distribution,
151 particle aspect ratio, fiber flexibility [22, 25] and physio/chemical properties of the substrate.
152 Water retention value (WRV) of the substrate directly impacts the apparent viscosity of a
153 suspension, affecting mixing and handling of the slurries [26]. For example, pretreated corn
154 stover (PCS) slurries are considered “pourable” when yield stresses are at or below ~10 Pa or
155 ~10% insoluble solids [3, 23]. Dilute acid PCS at 20% insoluble solids is a thick, paste-like
156 substance that can be molded and formed into shapes that remain even after the applied forces
157 are removed [23]. At even higher solids loadings (>30%), particles are not as lubricated because
158 of the lack of free water, resulting in increased friction due to particles interacting with both
159 water and other particles. At this point, the mixture can no longer be called a slurry because it is
160 unsaturated and acts more like a wet, granular substance. Substances with these varied
161 rheological properties present many unique challenges in materials handling throughout a
162 conversion process, particularly when continuous, industrial-scale processes are desired.

163 Several rheological models of interest, like the Bingham, Herschel-Buckley, Power Law,
164 Wildemuth-Williams and Casson models [3, 8, 21, 24, 27], have been developed to describe the
165 non-Newtonian behavior of these types of systems, but discussion of these models is beyond the
166 scope of this paper.

167 Um and Hanley [8] analyzed rheological properties of high-solids (10-20% w/v)
168 enzymatically hydrolyzed slurries of the model cellulose feedstock Solka Floc, a delignified

169 spruce pulp. Commercially-available *Trichoderma longibrachiatum*-sourced enzymes (30
170 FPU/g cellulose supplemented with β -glucosidase) were evaluated at 10, 15 and 20% solids
171 loadings. The enzymatic suspensions exhibited a pseudoplastic behavior overall, with viscosities
172 ranging from 0.04 to 0.01, 0.23 to 0.03, and 0.29 to 0.04 Pa·s for substrate concentrations of 10,
173 15 and 20% (respectively) initial solids measured at 50 °C. As the hydrolysis progressed, a
174 decrease in viscosity was observed for all solids loadings (dropping by approximately half in 3
175 hours). Zhang et al. [18] showed the same trend with high-solids steam exploded corn stover.
176 Several studies using dilute acid-pretreated corn stover also observed a reduction in yield stress
177 (and therefore viscosity) as solids loadings in enzymatic hydrolysis decreased (Figure 3) [3, 21,
178 22, 24, 27].

179 Additionally, Roche et al. [3] found that at 20% solids, >40% conversion was necessary
180 for the slurry to become pourable. They also reported a distinct difference between PCS that was
181 enzymatically hydrolyzed as compared to PCS that was just diluted. The yield stress for diluted
182 PCS is higher by a full order of magnitude than that of hydrolyzed PCS at corresponding particle
183 volume fractions. Although specific mechanisms for this difference were not investigated, one
184 theory is that the enzymes alter the particles during hydrolysis, converting them from complex
185 networks of material with distinct liquid and solid phases, to a homogeneous slurry as the liquid
186 and solid phases become indistinguishable.

187 Particle size affects the rheological properties of the suspensions, directly impacting
188 mixing and pumping costs [27]. Viamajala et al. [24] found that smaller particle sizes resulted in
189 smaller apparent viscosities under equivalent conditions. Mechanical pretreatment is often
190 utilized to reduce particle size to make the rheological properties more favorable for other steps
191 downstream in the process. However, temperature and acid concentration in dilute acid

192 pretreatment directly affect yield stress of a slurry, possibly as a result of a reduction in particle
193 size, as well as enhancing enzymatic hydrolysis due to the modification of the surface chemistry
194 of the particles [21, 27]. While a reduction in particle size lowers viscosity, as well as increases
195 conversion efficiency, the manner in which the size reduction occurs is also important. Size
196 reduction via pretreatment provides better digestibility and a reduced yield stress as compared to
197 mechanical size reduction, which did not significantly impact either property [27]. In some
198 cases, the pretreatment, like dilute acid pretreatment, hydrothermal pretreatment or SPORL
199 (sulfite pretreatment to overcome recalcitrance of lignocelluloses) performed prior to the
200 hydrolysis step alters the structure of the biomass significantly so that liquefaction occurs
201 quickly upon addition of the enzymes and mixing can resume [28, 29]. However, in most cases,
202 the solid fraction is still a complex network of fibrous material [21, 24, 30]. Sufficient mixing is
203 required for timely hydrolysis of the biomass, and traditional mixing methods like stirred-tank
204 reactors with impellers require excessive power and shaking does not provide adequate mixing.
205 Several mixing alternatives are discussed in a later section.

206 The pulp and paper industry has long used additives to modify rheological properties of
207 lignocellulosic slurries [25]. Knutsen and Liberatore [31] found that the most effective additive
208 groups (in descending order) to reduce yield stress were surfactants, additives with polar head
209 groups, additives with hydrophobic tails, unmodified protein and polymers. CTAB (cetyl
210 trimethylammonium bromide) and CPCI (cetylpyridinium chloride), both surfactants, were two
211 of the most effective additives for reducing yield stress. Samaniuk et al. [25] used water soluble
212 polymers (WSPs) like carboxymethyl cellulose (CMC), polyethylene oxide (PEO) and
213 polyacrylamide (PAM), to modify rheological properties of lignocellulosic slurries. Additives
214 like CMC reduced the friction between cellulose surfaces, making it easier to mix high-solids

215 suspensions. The addition of 2% CMC reduced the yield stress by ~67% from 55 kPa to ~18
216 kPa. A four-fold increase in CMC resulted in reducing by another 50%. They also found that a
217 lower degree of substitution for CMC had a positive impact on the yield stress; however, this
218 trend was more apparent at higher CMC loadings. Furthermore, a reduction in yield stress was
219 observed as the molecular weights of the WSPs increased up to a certain point. For example,
220 yield stress decreased with the addition of 600 kDa, as well as 2000 kDa, PEO, but no further
221 change in yield stress was observed with the addition of 7000 kDa PEO. Several other additives
222 were screened by monitoring the reduction in torque as measured by a torque rheometer to
223 determine whether they warranted further investigation. Fly ash and microcrystalline cellulose
224 were evaluated as possible additives, but their impact was limited. The surfactant Polysorbate 80
225 reduced the yield stress by 36% but required high concentrations (10%). Guar gum,
226 hydroxypropyl methyl cellulose (HPMC), a guar gum-xanthan gum mixture and a guar gum-
227 HPMC mixture were all more effective than CMC, where guar gum and the two mixtures
228 containing guar gum resulted in the highest reduction in torque (~80%). The addition of
229 additives may be costly, but like the pulp and paper industry, it may become economically
230 feasible to utilize such methods of modification for high-solids conversion processes. It is
231 important, however, that these additives be as inexpensive as possible and do not negatively
232 impact the conversion process by inhibiting the hydrolytic enzymes or fermentative organisms.
233

234 **4. Impacting Enzymatic Hydrolysis Rate and Extent**

235 The term “lignocellulosic biomass” refers to many different types of biomass, including
236 forestry and agricultural residues (woody biomass, straw, stover), fermentation by-products
237 (DDGS) and dedicated energy crops (grasses), just to name a few. Each type of lignocellulosic

238 material is slightly different in regards to composition, resulting in unique challenges in the
239 enzymatic hydrolysis step of the conversion process. The following sections are organized based
240 on various aspects in need of consideration during the conversion of lignocellulose and highlight
241 some of the challenges and breakthroughs associated with enzymatic hydrolysis performed at
242 high-solids loadings for different types of biomass. It is important to note that while each of
243 these processing approaches are discussed individually, it is often difficult to separate out the
244 combined effects of multiple process conditions.

245 Furthermore, when determining cellulose conversion, it is important to note that the
246 standard method of calculating conversions as described by [32] can grossly overestimate actual
247 conversion for high-solids systems. In some instances, conversions can be overestimated by up
248 to 36% [5]. Determining cellulose conversion in high-solids systems can become very
249 complicated, but several studies have proposed new methods for determining cellulose
250 conversion [5, 33, 34] under these high solids operating conditions. The standard method for
251 conversion calculations typically compares the amount of glucose measured in the hydrolyzate
252 (the liquid fraction) to the potential glucose found in the biomass (the solid fraction). This
253 method requires the assumption that all components have a consistent density throughout the
254 reaction and that it is approximately equal to that of water. As solids loadings increase, this
255 assumption no longer remains valid, resulting in overestimated conversions.

256

257 **4.1 Biomass Processing**

258 Enzymatic hydrolysis is an intermediate step in the conversion process, and while
259 producing high sugar yields is favorable, the resulting hydrolyzate must be subsequently capable
260 of supporting fermentative organisms while they produce biofuels. Some of the more expensive

261 steps in substrate preparation are washing the substrate following pretreatment and detoxifying
262 the hydrolyzate produced during enzymatic hydrolysis. It is likely that for industrial processes
263 unwashed, whole slurries (liquid + solids) from pretreatment will be used in enzymatic
264 hydrolysis [2], indicating a need for robust enzymes capable of maintaining their activity in the
265 presence of possible inhibitors and degradation products or developing pretreatments that do not
266 produce such products. Furthermore, the cost of hydrolyzate detoxification alone can be up to
267 22% of the total ethanol production cost [35].

268 Several studies have investigated the effects of eliminating washing and/or detoxifying
269 steps in the lignocellulose conversion process, with some promising results. Hodge et al. [2]
270 studied the effects of soluble and insoluble inhibitors on enzymatic hydrolysis by comparing the
271 glucose yields produced from a washed pretreated substrate (which introduces only potentially
272 insoluble inhibitors into the hydrolysis reaction since all soluble inhibitors are washed away) and
273 an unwashed whole slurry substrate (which introduces both potentially soluble and insoluble
274 inhibitors to the hydrolysis reaction). However, to maintain the high-solids loading and modify
275 the pH, the solid and liquid fractions were separated, the liquid fraction pH was adjusted, and the
276 two fractions were combined. Should the whole slurry be used at the industrial scale (as this
277 study states in its rationalization for this work), this method of pH modification may not be
278 feasible. This challenge is just one of many that must be solved prior to implementing a
279 complete conversion process. Regardless, this study utilized an insoluble solids loading of 5-
280 13% (~9-24% total solids loading) and relatively low enzyme loadings (<20 FPU/g cellulose).
281 Based on the glucose production from hydrolysis, the authors suggested that the limitations due
282 to mass diffusion are more prevalent than the sugar inhibition beyond a specific solid content.
283 For instance, sugar inhibition would result in a “leveling-off” of the hydrolysis rate, much like

284 what would be seen in a typical hydrolysis curve. However, a sharp decrease in the hydrolysis
285 rate was reported here. Using the washed substrate, this decrease is not prevalent until ~20%
286 insoluble solids loadings are reached, where convective mixing and available water are
287 negligible, likely indicating the point of mass transfer limitations. This decrease occurs at much
288 lower solids loadings (<10% insoluble solids) for unwashed substrate, indicating that the soluble
289 components contributed to a higher rate of enzyme inhibition or limited mass transfer by
290 reducing the amount of water available for reaction. (Further discussion on the restriction of
291 water can be found in Section 4.4 Solids Effects.)

292 Pristavka et al. [36] also conducted enzymatic hydrolysis studies with SO₂-catalyzed
293 steam exploded willow. These studies were concerned with simplifying the conversion process
294 by neglecting to wash the pretreated willow between the pretreatment and hydrolysis steps and
295 eliminating mechanical stirring of the biomass slurry. The reason for eliminating the washing
296 step was two-fold. First, less water would be used in the conversion process, making the process
297 more economical and more environmentally friendly. Secondly, washing usually leads to the
298 solubilization and removal of a significant portion of sugars. These sugars ultimately end up
299 accumulating in wastewater, resulting in an expensive processing step to recover them and/or
300 treating the water. The high-solids loadings (up to 25% ODM (organic dry matter)) used in this
301 study would make mechanical stirring of the slurry extremely energy intensive, so it was
302 removed. With these process modifications, a lower degree of conversion was observed as
303 compared to biomass that was washed prior to hydrolysis (53% vs. 74%). However, the degree
304 of cellulose conversion increased to >95% when the pH of the unwashed, pretreated willow was
305 adjusted with solid NaOH to the optimal pH of the enzymes. The significant increase in
306 conversion following pH adjustment highlights the importance of maintaining optimal hydrolysis

307 conditions for the enzymes, even if that means finding new, inexpensive and less resource-
308 intensive methods of doing so.

309 Lu et al. [37] investigated the effects (post-pretreatment) washed substrate had on
310 enzymatic hydrolysis and fermentation. Using steam-exploded corn stover, substantial
311 differences in conversion efficiencies were not observed for washed and unwashed substrates up
312 to a solids loading of 30% (w/w). However, closer examination of the conversion calculations
313 revealed differences between washed and unwashed substrates, since conversions were based on
314 water insoluble solids and not total solids content. (Essentially the denominators were different
315 for the two treatments.) Additionally, the pH of the unwashed corn stover was not adjusted prior
316 to addition of enzymes and buffer at pH 4.8. Cellulose conversion remained fairly consistent
317 (70-75%) for all solids loadings, although glucose content was higher for the washed substrate
318 than the unwashed substrate. Ethanol production was also independent of solids loading (up to
319 30% w/w) for the water-washed corn stover, reaching 92-94% of theoretical yield. However, the
320 results were quite different for the unwashed substrate. At the lower solids loadings studied (10-
321 15% w/w), ethanol production fell to 88% and 86%, respectively, and decreased as the solids
322 loading increased, until no ethanol could be measured ($\geq 25\%$ solids loading). The levels of
323 acetic acid and furfural measured at the higher solids loading reached inhibitory concentrations.
324 Inclusion of the water-washing step following pretreatment appears to eliminate the need for
325 another costly detoxification step following enzymatic hydrolysis for steam-exploded corn
326 stover.

327 In contrast to this study, others report contradicting results regarding the wash step [35,
328 38]. Lau et al. [35] reported that when AFEX-pretreated corn stover was fermented following
329 enzymatic hydrolysis at 18% (w/w) solids loading, the ethanol yield of ~93%, even though the

330 solids loading during hydrolysis and glucose concentration before fermentation were similar to
331 those reported in Lu et al. [37] who reported a 68% ethanol yield. While these results are so
332 different, it should be noted that different pretreatments, as well as fermentative organisms were
333 used (*E. coli* vs. *S. cerevisiae*, respectively), making it difficult to directly compare these
334 fermentation results. However, Lau and Dale [38] achieved higher ethanol production rates
335 fermenting unwashed substrates (~0.17 g/L/hr as compared to 0.12 g/L/hr for washed substrate)
336 with *S. cerevisiae* 424A (LNH-ST) (a genetically modified strain for improved xylose
337 fermentation), suggesting that the elimination of the washing step following pretreatment, and
338 with no adjustments made to the pH prior to hydrolysis, is beneficial for fermentation under the
339 conditions examined in this study. Ethanol concentration from unwashed substrate was 40 g/L
340 (no data given for washed substrate). Xylose metabolism from the genetically modified strain is
341 likely the largest contributing factor to the discrepancy in reported ethanol yields, but it was also
342 reported that the this strain of *S. cerevisiae* performed similarly on washed substrate as compared
343 to unwashed substrate. This study suggests that the washing step can be eliminated without any
344 loss in ethanol yield. Contradictory results indicate the need for further study of this issue, or at
345 the very least, optimization studies under specific process conditions.

346 In another study, LHW-pretreated sweet sorghum bagasse was hydrolyzed at 15-30%
347 solids (w/v) with either 20 or 30 FPU/g glucan cellulase [39]. Washing the substrate prior to
348 hydrolysis also did not improve the conversion rates. Washed substrate yielded 63.2 g/L of
349 sugar, whereas the unwashed substrate resulted in a sugar concentration of 66.1 g/L. It was
350 suggested, although not verified, that the washing step actually removed some of the smaller
351 cellulose particles that may have been easier to hydrolyze than larger cellulose particles.

352 The inconclusive results of these studies illustrate the complexity of defining appropriate
353 processing conditions that work in all situations. Operating conditions must be chosen carefully
354 in order to realize the full potential of using lignocellulose as a valuable energy source. Table I
355 illustrates the wide variety of operating conditions that have been studied with regards to high-
356 solids loadings enzymatic hydrolysis. Depending on various factors, like substrate choice,
357 pretreatment conditions and hydrolysis conditions, it may be possible to eliminate certain steps
358 like washing pretreated substrate or detoxifying hydrolyzate prior to fermentation, thus
359 simplifying the overall conversion process. However, elimination of these steps may present
360 new problems that must be solved. For instance, should the washing step following pretreatment
361 be eliminated, it may be necessary to adjust the pH in another manner so the hydrolytic enzymes
362 can work most effectively.

363

364 **4.2 Feeding Strategies**

365 Fed-batch feeding schemes have been investigated as an alternative method of achieving
366 high-solids loadings in enzymatic hydrolysis [1, 26, 45, 46] because of some of the advantages it
367 offers over single feeding schemes. For instance, the initial viscosity is lower, so diffusion and
368 mixing limitations can be minimized or altogether avoided. A fed-batch feeding regime also
369 allows time for the slurry to liquefy before adding additional solids, which maintains a level of
370 free water that is available for the reaction process and for diffusion (away from the enzymes) of
371 potentially inhibitory products that result from the hydrolysis reaction. However, when a fed-
372 batch approach is selected, one must consider how and when to add substrate, as well as
373 enzymes, to the reaction in order to maintain high rates of conversion. Table II illustrates the
374 variety of substrate and enzyme application rates used in fed-batch studies.

375 Hodge et al. [1] conducted a study in which the fed-batch approach was utilized in order
376 to achieve a final insoluble solids content of 15% (w/w) (equivalent to a 25% (w/w) initial solids
377 loading). This solids loading was the upper limit of unhydrolyzed pretreated corn stover that
378 could be effectively mixed in the stirred tank reactors (STRs) available to the researchers. High
379 cellulose conversion (>80% cellulose conversion) was reported; however, the reaction time was
380 more than double the typical hydrolysis reaction time (168 hrs vs. 72 hrs). The extended time
381 problem may be overcome through the use of higher enzyme loadings or enzymes that can
382 tolerate higher sugar concentrations. The enzyme loading used in this study was 10.7 FPU/g
383 cellulose, a relatively low loading, and it was applied proportionally with each addition of
384 substrate. A study conducted by Yang et al. [46] obtained a similar cellulose conversion
385 (70.6%), with a higher solids loading (30%), an enzyme loading almost twice (20 FPU/g
386 cellulose) that used in the former study and with a much shorter reaction time (30 hrs). Both
387 studies attribute the high conversion rate, at least in part, to the fact that the substrates were
388 washed prior to hydrolysis, possibly eliminating any potential inhibitory products that resulted
389 from the pretreatments. The latter study also supplemented fresh enzyme with each addition of
390 new biomass, which increased the final enzyme loading from 10 to 15 FPU/g cellulose. The
391 fresh enzyme may have also enhanced the glucose yield, replacing the enzyme that may be non-
392 productively bound to the lignin or deactivated by extended hydrolysis times.

393 Zhang et al. [52] studied another fed-batch approach for the conversion of NaOH-
394 pretreated sugarcane bagasse and wheat straw. Pretreated biomass was fed into the reactor at
395 9%, 8%, 7%, and 6% solids over the course of 48 hrs to achieve a final solids loading of 30%
396 (w/v). All enzymes were added with the first addition of lignocellulose. Glucose conversion
397 from wheat straw reached a maximum (~60%) after the first feeding, but decreased with each

398 successive feeding. The higher rate of conversion was likely due to the low solids loading and
399 high enzyme loading at the beginning of the reaction. With each successive feeding, the
400 enzyme: substrate ratio decreased. After 72 hr of hydrolysis, the conversion began to level off,
401 resulting in a final glucose conversion of 39%. A slightly different conversion profile was
402 observed with the bagasse. The conversion continued to increase over the course of the
403 hydrolysis reaction, with the exception of the last feeding time (6% solids at 48 hr). The final
404 feeding resulted in a sharp decrease in conversion, but it recovered within 24 hr following the
405 feeding, leading to an increase in conversion over the batch. The final glucose conversion of the
406 sugarcane bagasse was 55%. Differences in the way the pretreatment affected the lignocellulose
407 may have led to the different glucose yields between the two substrates. It was reported that the
408 pretreatment caused the surface of the two substrates to become rough and fragmented as lignin
409 was removed, allowing for better access to the cellulose; however, the bagasse appeared to have
410 a rougher, more fragmented surface than the wheat straw. Following 144 hr of hydrolysis, the
411 surfaces were relatively smooth as compared to the start of the hydrolysis.

412 Wang et al. [39] considered the use of a fed-batch feeding scheme. Initially, the reactors
413 were charged with half of the final solids loading, followed by two additional feedings at 24 and
414 48 hr of one-fourth of the final solids loading. The system containing 30% solids achieved the
415 highest final sugar concentration with nearly 115 g/L. Even with the fed-batch system, the
416 conversion decreased with increasing solids loadings; however, the conversion of the 30% solids
417 reaction was only 5% less than the systems at 15% and 20% solids (55% vs. ~60%,
418 respectively).

419 Fed-batch was utilized by Ma et al. [55] to achieve a 25% (w/v) solids loading. Enzymes
420 were added either all at once at the beginning of the reaction or with each addition of the dilute

421 acid pretreated cassava bagasse. At this solids loading, the batch reaction reached ~50%
422 conversion, whereas the fed-batches with a single enzyme addition and multiple enzyme
423 additions achieved ~75% and 84% conversion, respectively. These results are similar to those
424 reported in other fed-batch studies [1, 46], indicating that under the right conditions fed-batch
425 systems may be a plausible solution for achieving higher conversion rates for hydrolysis
426 performed at high-solids loadings.

427 Rosgaard et al. [26] investigated several different regimes for batch and fed-batch
428 hydrolysis, including variations of sequential addition of substrate as well as substrate plus fresh
429 enzyme. The addition of fresh enzyme with each substrate addition maintained a constant
430 enzyme:substrate ratio throughout the whole reaction, as opposed to the other fed-batch feeding
431 schemes where all the enzyme was added in one application. In these cases, the effective
432 enzyme:substrate ratio decreased with each subsequent addition of substrate. Not surprisingly,
433 the fed-batch schemes that received the full enzyme application at the start of the reaction
434 produced higher glucose yields during the first few hours as compared to the fed-batch reactions
435 that received fresh enzyme with each substrate addition. However, the extent of the hydrolysis
436 reaction was not affected by the method of enzyme application as the final glucose
437 concentrations were not different for the fed-batch reactions with and without additional enzyme
438 applications (62-67 g/L). Furthermore, lower viscosity is often touted as an advantage of fed-
439 batch systems over batch systems because mixing becomes easier as viscosity decreases. The
440 viscosities of the fed-batch systems in this study were lower than in the batch systems, but no
441 benefits were observed in regards to glucose production as the batch system at 15% solids
442 resulted in higher glucose production (78 g/L) after 72 hr hydrolysis. Final glucose
443 concentrations of the fed-batch systems, though, were impacted by each addition of substrate.

444 Hydrolysis rates decreased and never fully recovered, resulting in lower final yields than the
445 batch systems.

446 Additionally, Chandra et al. [45] reported on a fed-batch approach at a moderate solids
447 loading that did not perform as well as a single stage feeding approach. The total solids loadings
448 achieved for both feeding schemes was 10%. Two enzyme loadings were tested (5 and 60
449 FPU/g cellulose), and at both loadings, the batch reaction produced the higher yields,
450 approximately 66% and 90% for steam-pretreated corn stover, respectively. However, when the
451 solids are fed at 24 hr intervals, the respective yields are lower (approximately 55% and 80%)
452 and the hydrolysis rates slower. The authors suggest these reductions in yields and rates are the
453 result of non-productive binding of enzyme to xylan or lignin fractions of the substrate or the
454 inability of the enzyme to desorb from partially hydrolyzed substrate and find accessible
455 cellulose sites in the fresh substrate. Free protein measurements taken at 72 hr indicate that 50-
456 70% of the cellulase was still adsorbed to the substrate for both enzyme loadings, while the
457 cellulose conversion ceased. The lower hydrolysis rate at the higher enzyme loading seems to
458 indicate that the enzymes are saturating the accessible cellulose sites, thus reaching a maximum
459 hydrolysis rate that is lower than that of the batch reaction when all the accessible cellulose sites
460 are available at once.

461 The results of fed-batch feeding schemes are currently still inconclusive, as indicated by
462 the preceding studies, making the decision to use a fed-batch approach unclear. Many
463 advantages are realized regarding the use of fed-batch systems, but questions persist. For
464 instance, at what point in the reaction should subsequent additions of substrate be applied to
465 maintain a high rate of conversion? Should enzymes be added in a single application, as a
466 supplement to the original application, or proportionally to the substrate? Does the benefit of

467 reduced viscosity make a difference in energy consumption during the conversion process to
468 overcome the potentially reduced sugar yield that may result from the fed-batch as compared to
469 the batch system?

470

471 **4.3 Effects of Enzyme Synergism**

472 Enzymatic hydrolysis, especially at high-solids loading, has been identified as the largest
473 impediment to achieving high yields in a timely manner in the lignocellulose to ethanol
474 conversion process, mainly because a significant portion of sugars produced are in oligomeric or
475 polymeric form, which cannot be used in the fermentation process. Several studies have
476 investigated this issue from the perspective of the enzyme (Table I), experimenting with enzyme
477 supplementation (in addition to cellulase) and alternative organism sources for cellulase [38, 47-
478 49]. Supplementing cellulase with β -glucosidase has long been used to minimize end-product
479 inhibition of the cellulase and achieve higher conversions. Lau et al. [48] investigated the use of
480 several different enzymes other than cellulase and β -glucosidase to enhance the conversion of
481 lignocellulose. Their enzyme cocktail included xylanase and pectinase to target the
482 hemicellulose that acts as a barrier to cellulose if not removed during pretreatment. The focus of
483 this work was on the fermentation step, so the details regarding the enzymatic hydrolysis are
484 limited. However, the hydrolyzates produced from AFEX-pretreated corn stover with these
485 enzyme cocktails were able to produce 40 g/L (5.1% v/v) of ethanol with *Saccharomyces*
486 *cerevisiae*.

487 Another study investigated the effects of supplementing the typical cellulase and β -
488 glucosidase enzyme cocktail with xylanase on the hydrolysis of steam-exploded barley straw
489 [50]. The addition of the xylanase to the enzyme mixture enhanced the conversion rate of the

490 cellulose, especially at low solids loading and early in the hydrolysis reaction. Conversion at
491 higher solids loadings may be reduced by the higher concentration of xylooligomers produced
492 with the addition of xylanases, as has recently been shown [15]. However, the xylanase used in
493 the supplementation study did contain some β -xylosidase activity, which, if present, might
494 counteract the inhibition caused by xylooligomers. The positive effects of the xylanase addition
495 reported in this study support the idea that overall enzyme loadings could be reduced if better
496 conversion is achieved by incorporating an array of different enzymes. However, a different
497 study conducted by Di Risio [44] also evaluated various enzyme cocktails made from
498 commercially-available enzyme solutions. All three cocktails assessed consisted of the same
499 base solution: cellulase and β -glucosidase. Each solution was supplemented with a third
500 commercial enzyme solution with different active components: cellulase + xylanase, cellulase +
501 xylanase + β -glucosidase, and xylanase. The highest glucose yields (44%) resulted from the
502 enzyme cocktail consisting of the base solution supplemented with the commercial solution
503 containing cellulase + xylanase + β -glucosidase activity. Surprisingly, the enzyme solution
504 supplemented with the enzyme promoted as a “xylanase” actually yielded significantly less
505 xylose than the other two enzyme solutions (39% as compared with 54% and 85%). However,
506 there is no indication that the xylanase activity of this commercial product was independently
507 verified prior to use. Glucose yields ranged from 32%-42%.

508 Taking it a step further, another group studied the effects of various addition schemes and
509 enzyme loadings using an enzyme cocktail containing cellulase, β -glucosidase and xylanase on
510 the hydrolysis of mixed hardwood chip pulps [42]. The enzyme cocktails consisted of fungal
511 cellulase (C), xylanases (X) and β -glucosidase (B) solutions mixed in the ratio of 10:3:3 (by
512 volume). The mixtures were added to the substrate in the following manners: (1) cellulase,

513 xylanases and β -glucosidase was mixed with substrate at the desired solids loading (CXB); (2)
514 cellulase was added to 5% solids, pressed or filtered to obtain the desired solids loading, and
515 hydrolyzed for a period of time before the xylanases and β -glucosidase mixture was added
516 (C+XB); and (3) half of the cellulase was added to 5% solids, pressed or filtered to obtain the
517 desired solids loading, and hydrolyzed for a period of time before the cellulase (half dose),
518 xylanases and β -glucosidase mixture was added (C+CXB). With the CXB mixture, a decrease in
519 conversion was observed with an increase in solids loading. Enzyme loading also plays an
520 important role in the optimization of biomass conversion. For example, with the CXB enzyme
521 mixture, the difference in sugar yields decreased with increased enzyme loadings. At 40 FPU/g
522 solids, conversion decreased from 70% to 68% for 5% and 20% solids loading, respectively,
523 which represents no significant difference in conversion. However, at 5 FPU/g solids,
524 conversion decreased from 40% to 19% for 5% and 20% solids loadings, respectively. The
525 authors hypothesized the decreased conversion was the result of ineffective mixing of the
526 enzyme mixture with the substrate as the solids loadings increased. Based on this hypothesis, the
527 authors added the enzyme to a low solids mixture, allowing time for the enzymes to adsorb to the
528 substrate, before filtering off 80% of the liquid to obtain 20% solids loadings. Enzyme activity
529 was tested following filtration to determine whether any enzyme was lost during this process.
530 Cellulase activity registered at 80% of the original activity, whereas only 20% of the xylanases
531 activity was retained. This observation resulted in the modified application of the enzyme
532 mixture (C+XB). At 20% solids and 20 FPU/g solids, sugar conversion increased from 44% for
533 the CXB mixture to 59% for the C+XB mixture. Sugar concentrations increased from 84 g/L to
534 114 g/L. This modified enzyme application process was also beneficial at low solids loadings
535 (5%), increasing conversion from 19% with CXB to 38% with C+XB. Taking this enzyme

536 application process one step further, additional cellulase was added with the xylanases and β -
537 glucosidase mixture (C+CXB). In this instance, although the sugar concentration increased to
538 121 g/L glucose (63% conversion), the conversion at 20% solids was similar to that at 5% solids
539 at all enzyme loadings tested. These experiments indicate the importance of determining enzyme
540 mixtures and application schemes that provide the optimal sugar yields and concentrations for
541 the conversion process.

542 Along with the feeding scheme and the enzyme loading, the type of enzyme used can
543 have a significant impact on the liquefaction of biomass. The term “cellulase” can refer to a
544 wide variety of enzymes, and commercially available enzymes can often be a crude mixture of
545 enzymes (i.e. *T. reesei* cellulase that is commonly used in hydrolysis studies). To be more
546 specific, for example, the *T. reesei* “cellulase” can refer to a mixture of cellobiohydrolases
547 (CBH), endoglucanases (EG), xylanases (XYLs), and β -glucosidase, among other enzyme
548 components. Using an array of CBHs, EGs, XYLs and a β -glucosidase, both individually and in
549 combination, Sjizarto et al. [30] assessed the enzymes on their ability to liquefy hydrothermally
550 pretreated wheat straw. For the *T. reesei* components, it was determined that the EGs (especially
551 Cel5A) were the most important in liquefying lignocellulose. This enzyme alone reduced the
552 viscosity of the slurry by nearly 90%. The CBHs and XYLs had little to no effect on the
553 viscosity, even though the sugar production was similar to that of some of the EGs.
554 Furthermore, a mixture of enzymes produced the highest sugar yields, even though the viscosity
555 was reduced by only about 82%, indicating that the amount of sugar hydrolyzed is not the main
556 factor in reducing viscosity, but that the sites at which the polysaccharides are cleaved is more
557 important.

558 Since enzymes play such a vital role in the conversion of lignocellulose, much of the
559 process integration depends on these biological catalysts. For instance, a balance must be struck
560 between the enzyme loading used and enzyme cost. High enzyme loadings not only increase the
561 total cost, but as discussed in the introduction, studies suggest that enzymes are overcrowding
562 accessible cellulose chains, thus reducing the rate at which cellulose is hydrolyzed. One such
563 study was conducted by Olsen et al. [58]. At a solids loading of 29% (w/w) pretreated corn
564 stover, a range of enzyme loadings (5-83 FPU/g cellulose) were evaluated for hydrolysis yields.
565 At enzyme loadings >66 FPU/g cellulose, the hydrolysis curves started to coincide. It was
566 suggested that the lack of improvement in hydrolysis rate and conversion was due to the
567 substrate being completely saturated with enzymes bound to all the accessible sites. High
568 enzyme loadings also do not make sense economically. Based on a techno-economic model of
569 the bioethanol conversion process, an optimum total solids loading of about 20% with an enzyme
570 loading of 20 mg/g solids (8.8 FPU/g solids) was determined to produce the minimum ethanol
571 selling price with currently available, commercial enzymes [4]. This model evaluated the cost of
572 production at 2007 enzyme production costs (\$0.35/gal), as well as the enzyme production cost
573 projected by the Multi-Year Program Plan (MYPP) from the DOE's Office of Biomass Program
574 for 2012 (\$0.12/gal) [59]. At the lower enzyme production cost, solids loadings could
575 potentially be increased up to 26% and remain economically viable. In the time since this study
576 was published, the MYPP re-evaluated the cost of enzyme production and the current projection
577 for 2012 was fairly consistent with the "high" cost of enzyme production reported in the study at
578 \$0.34/gal of ethanol (2007\$). Under the assumptions made constructing this model, 20% solids
579 loading remains the maximum that is economically feasible for the ethanol production process.

580 Zhang et al. [43] evaluated enzyme loading to determine the effect it had on glucose
581 concentration. A 50% reduction in enzyme loading decreased the glucose concentration by only
582 21%. The implication of this observation is that enzyme loading can be optimized to provide the
583 maximum concentration at the lowest unit cost. For example, it may not be worth converting an
584 extra 5% of glucose if it accounts for ~15% of the total enzyme cost unless the return on the
585 extra glucose recovers the cost of the additional enzyme.

586 While the cellulase system of *T. reesei* is one of the most commonly studied enzyme
587 systems, other organisms also produce cellulolytic enzymes that could potentially impart
588 superior activity under certain conditions. Ingram et al. [53] compared the conversion
589 efficiencies of enzymes from two different organisms, *T. reesei* and a genetically-modified (for
590 increased cellulase production) strain of *Penicillium janthinellum*. Enzyme mixtures from both
591 organisms contained cellulases, β -glucosidases and xylanase activity. With the cellulase from *T.*
592 *reesei*, an increase in glucose concentration as biomass loading increased was observed for the
593 organosolv and the LHW-pretreated rye straw. After 48 hrs of hydrolysis at 17.5% solids, the *P.*
594 *janthinellum* cellulase converted 72% of the soda-pretreated rye straw. Higher enzyme loadings
595 of *P. janthinellum* cellulase were necessary to achieve the same level of conversion produced by
596 the *T. reesei* cellulase (27 FPU/g cellulose vs. 13 FPU/g cellulose); however, the *P. janthinellum*
597 cellulase appeared to be more tolerant to changes in pH. This study highlights the fact that the
598 conversion process is dependent on many factors, including, but not limited to, the type of
599 biomass, the conditions of the pretreatment, and the source of enzymes.

600 In another study partially purified cellulase from the thermostable *Geobacillus* R7 was
601 evaluated as an alternative cellulase source [47]. For short hydrolysis times (36 hr), the
602 *Geobacillus* cellulase was comparable to a commercial enzyme preparation. However, for

603 hydrolysis of pretreated prairie cord grass using this cellulase, the glucose recovery at 96 hrs for
604 solids loadings $\geq 10\%$ was between 46.2% and 48.7%. It does not appear that the solids loading
605 had much of an impact on conversion of the prairie cord grass; although the conversion of
606 cellulose into glucose utilizing the *Geobacillus* R7 cellulase was better than the conversion of the
607 pretreated corn stover at 27%-31%. *Geobacillus* R7 also has the added benefit of being
608 ethanologenic. During the hydrolysis, *Geobacillus* R7 produced a small amount of ethanol
609 (0.035 g/L) from the pretreated prairie cord grass, which has possible implications for
610 consolidated bioprocessing of lignocellulose materials. Subsequent fermentation of the
611 hydrolyzate with *S. cerevisiae* resulted in an ethanol production of 7.8 g/L (or 0.47 g ethanol/g
612 glucose) for the 20% solids loading of prairie cord grass.

613 Lastly, Matano et al. [60] engineered fermentative yeast to express three different types
614 of cellulase on its surface. This yeast was subsequently evaluated in SSF processes utilizing
615 25% (w/v) pretreated rice straw. Initially, a control yeast strain was supplemented with a
616 commercial cellulase (100 FPU/g biomass). This combination resulted in an ethanol yield of
617 80% and liquefaction after 72 hr. When combined with the modified yeast strain, the
618 commercial cellulase loading could be reduced to 10 FPU/g biomass and produce the same
619 ethanol yield (79%). Further study showed that a maximum ethanol concentration (43.1 g/L)
620 was obtained following a 2 hr liquefaction period prior to the addition of the modified yeast,
621 corresponding to an ethanol yield of 89%. Residual glucose was reduced by an order of
622 magnitude with the modified strain (16 g/L to 1.6 g/L). The authors hypothesized that the close
623 proximity of the cellulases on the surface of the yeast provided a synergistic effect that resulted
624 in an increased hydrolysis of cellulose. As commercial enzymes are still a relatively large
625 portion of the overall cost of the conversion process, the ability to reduce the commercial

626 enzyme loading and replace it with an organism capable of both the hydrolysis and fermentation
627 is very attractive.

628

629 **4.4 Solids Effect**

630 For conversion of lignocellulose into usable and valuable products, it makes economical
631 sense to utilize locally-available biomass, as shipping biomass over long distances greatly
632 reduces the beneficial impacts. Cara et al. [41] studied the conversion of olive tree pruning
633 biomass (consisting of leaves and thin branches) up to 30% (w/v) solids loadings. The final
634 glucose concentrations increased with increasing solids loading, achieving 61 g/L and 52 g/L
635 glucose at 30% solids loading of the liquid hot water (LHW) pretreated biomass and steam
636 exploded biomass, respectively. However, the conversions of the LHW-pretreated biomass
637 decreased nearly linearly from 76.2% at 2% solids to 49.9% at 30% solids. Conversions of the
638 SE-pretreated biomass held steady between 60% and 63% up to 10% solids loading before
639 decreasing to 39.6% at 30% solids. In a different study, the researchers also observed that the
640 glucose concentration decreased as the solids loading was increased beyond 10% solids for the
641 soda pretreated rye straw [53]. The overall conversion of cellulose decreased from ~65% to 40%
642 as solids loadings increased from ~10% to 17.5%. This result is not unusual, as most studies
643 performed at high-solids loadings sacrifice conversion for a more concentrated glucose product
644 [10, 29, 41].

645 Kristensen et al. [10] also studied four mechanisms that possibly contribute to the so-
646 called solids effect: (1) compositional and substrate effects, (2) product inhibition, (3) water
647 concentration, and (4) cellulase adsorption. These mechanisms were studied with filter paper,
648 which is essentially a pure cellulose substrate. The researchers observed the same decreasing

649 trend in conversion as solids increased using the filter paper, much like that observed with
650 lignocellulose. Therefore, it was concluded that lignin, which is absent in filter paper, is likely
651 not the reason for the solids effect. Study of the second mechanism, product inhibition, resulted
652 in significantly different conversions after 48 hours of hydrolysis for 5% DM and 20% DM
653 (64.5% vs. 38.6% or 30 g/L vs. 86 g/L, respectively). However, the final conversions for these
654 solids loadings with an additional 50 g/L glucose added resulted in fairly similar conversions
655 (29.7% and 26.3% or 64 g/L vs. 109 g/L for 5% DM + 50 g/L glucose and 20% DM + 50 g/L
656 glucose, respectively). This experiment did not elucidate the exact reason for the observed
657 similar conversions, but two hypotheses were offered. It was suggested that other components in
658 the hydrolysis mask the product inhibition or that enzymes are inhibited similarly once a certain
659 glucose concentration is reached.

660 Kristensen et al. [10] next attempted to quantify the effects of water on the hydrolysis
661 reaction. Water content was decreased by 25% and replaced by oleyl alcohol. The alcohol
662 allowed the viscosity of the slurry to remain constant, thus removing the effects of the viscosity,
663 while the water to solids (or enzyme) ratio was altered. With this decrease in water, a 5%
664 decrease in glucose yield was observed. However, increasing the solids content from 20% to
665 25% (which is essentially equivalent to a 25% reduction in water), typically decreases glucose
666 yields by $\geq 12\%$. The authors argue this discrepancy in glucose reduction indicates that lower
667 water content is apparently not the limiting factor responsible for the solids effect.

668 Lastly, cellulase adsorption was investigated as a possible source of the solids effect [10].
669 Cellulase adsorption to filter paper was determined by measuring the total nitrogen content of the
670 biomass after 24 hr of hydrolysis. The amount of adsorbed cellulase measured was halved (40%
671 to 17%) as solids loading increased from 5% to 25%. At the same time, conversion was reduced

672 from ~60% to <50%. A strong correlation between decreasing adsorption and conversion was
673 observed, indicating that cellulase is not effectively adsorbing onto cellulose causing a decrease
674 in yield. The authors hypothesize that increasing concentrations of glucose and cellobiose inhibit
675 the adsorption of enzymes. Knowledge of the mechanisms of high-solids product inhibition and
676 the mechanisms of high-solids enzyme adsorption inhibition can provide the key to improving
677 the overall conversion process, thus unlocking the full potential of high-solids conversions.

678 In contrast to the previous study, Roberts et al. [56] investigated the interactions of water
679 with biomass at high-solids loading without maintaining a constant viscosity. Time domain
680 NMR was used to measure the transverse (or spin-spin) relaxation times (T_2) of protons in water
681 molecules to indicate the extent of water constraint (or degree to which water is tightly bound to
682 biomass). Essentially, the nuclei of water molecules that are tightly bound have a shorter
683 relaxation time than nuclei that are less tightly bound. By measuring these relaxation times,
684 constraint can be determined. It was found that water was more tightly bound as solids loadings
685 increased, suggesting that an indirect relationship between water constraint and yield exists.
686 However, the relaxation time of the primary bound water (water that interacts directly with the
687 surface of the cellulose) was constant regardless of the solids loading. Interactions at the water-
688 solids interface appear to remain constant, suggesting the chemistry at the surface of the
689 cellulose does not change as water content changes. These results further suggest that the water
690 primarily interacts with the cellulose, and the impact of the solute is minimized. However, these
691 studies were conducted with bacterial cellulose, a substrate that is essentially pure cellulose. It is
692 unclear whether cellulose derived from pretreated lignocellulose would interact with water in a
693 similar manner or to what extent the type of pretreatment may affect these cellulose-water
694 interactions. With the addition of excess glucose or mannose to 5% solids, the hydrolysis rate

695 reduced to one similar to 15% solids loading. The authors hypothesize that the negative effects
696 on the hydrolysis rate are caused by water constraint as opposed to the monosaccharides
697 impacting the enzyme activity. It is also possible that the lack of available water limited the
698 uniform distribution of synergistic enzymes, thus hindering the hydrolysis rate. Also, in contrast
699 to the previous study, the results presented in this study indicate that water (or the lack of it) has
700 a great impact on the overall hydrolysis rate. Even though the addition of oleyl alcohol in the
701 former study reduced the water content in the reaction, the constant viscosity helped maintain
702 adequate mixing and therefore did not limit the diffusion of enzymes throughout the suspension.
703 While these studies draw conflicting conclusions on the effect of water on lignocellulose
704 conversion, they do highlight the need for effective mixing. Adequate mixing was provided in
705 the former study, even with a low water: substrate ratio because of the low viscosity afforded by
706 the addition of alcohol, whereas the latter study simply reduced the water: substrate ratio without
707 regard for the viscosity. These studies also highlight the difficulty of quantifying and assigning
708 the challenges of operating at high solids to any one factor (lack of water, high viscosity,
709 adequate mixing, etc.) when all these factors are so interrelated.

710

711 **4.5 Effect of Viscosity on Mixing**

712 High viscosity of high-solids slurries is another hurdle that must be overcome. Much of
713 the previous discussion (i.e. effects of enzymes on liquefaction and solids loadings) also affects
714 the rheology, but this section discusses specific viscosity modifiers and their effects on
715 enzymatic hydrolysis. Ineffective mixing increases the limitations associated with mass transfer,
716 including removal of local inhibitors and hydrolysis products and transfer of heat throughout the
717 reactor. The pulp and paper industry has long been using viscosity modifiers to enhance the

718 processability of fibrous slurries [31], much like the types of slurries produced by lignocellulose
719 materials prevalent in the conversion to biofuels and biochemicals. One study [31] investigated
720 the use of 18 different chemical additives and evaluated the effects on the slurry rheology and
721 hydrolysis rates. Several surfactants added to lignocellulosic slurries at 2% (w/w), including
722 CPCI, CTAB, sodium dodecylbenzene sulfonate (NaDBS) and sodium dodecyl sulfonate (SDS),
723 positively affected the rheological properties of the slurry by reducing the viscosity by nearly
724 four-fold as compared to the viscosity of the unmodified slurry. Although slight decreases in the
725 extent of the hydrolysis reactions were observed, only the CPCI and the CTAB did not reduce
726 hydrolysis rates. Additionally, Ma et al. [55] tested the surfactant Tween-80 and found that it did
727 not produce a significant increase in conversion at a 10% solids loading to warrant its use.
728 However, at 25% solids loading, the addition of the surfactant (2 g/L) increased cellulose
729 conversion by 30%. Contrary to what Kristensen et al. [10] said, the inhibition caused by non-
730 productive binding of the enzyme to lignin does not seem to have as large of an effect at low
731 solids as it does at high solids. These results show some promise in modifying viscosity
732 properties of lignocellulose slurries; however, more work is warranted to understand the
733 mechanism by which these surfactants work, as well as determining the economical value of the
734 use of such additives.

735 Another approach to reducing viscosity is to raise the temperature at which the hydrolysis
736 reaction takes place [61]. In order to work at higher temperatures, enzymes that can tolerate the
737 increased temperatures must be used. It has been shown that EGs from more thermotolerant
738 organisms worked better at reducing the viscosity of a lignocellulose slurry, while other types of
739 enzymes appeared to have little effect [61]. *T. aurantiacus* proved to be more thermotolerant
740 than *A. thermophilum*, as the *T. aurantiacus* EG continued to reduce the viscosity at temperatures

741 up to 75°C. *A. thermophilum* enzymes were less active above 65°C, resulting in a reduced effect
742 on the viscosity. The ability to use alternate sources of cellulase enzymes illustrates the number
743 of reaction condition variables (i.e. temperature, components in enzyme cocktail, and solids
744 content in slurry) open to modification.

745 The method of mixing the slurry can also have a substantial impact on the conversion of
746 lignocellulose. For example, Zhang et al. [43] observed a significantly reduced liquefaction time
747 when comparing hydrolysis at high solids (17-20% w/w) performed in shake flasks with a lab-
748 scale peg mixer. Peg mixers are commonly used in the pulp and paper industry, which routinely
749 utilizes solids loadings up to 35% [43]. (Readers are referred to the section entitled “Reactor
750 design for enzymatic hydrolysis at high solids” for more details on the peg mixer.) Liquefaction
751 occurred after 1 hr of hydrolysis in the peg mixer, whereas the shake flask required 40 hr. The
752 decrease in liquefaction time can most likely be attributed to the effective mixing provided by
753 the peg mixer and the breaking down of the large fiber network that tends to occur as solids
754 loadings surpass 8%. At 20% (w/w) solids loadings, hydrolysis performed in the peg mixer
755 resulted in 144 g/L and 158 g/L of glucose from unbleached hardwood and Organosolv
756 pretreated poplar, respectively. These concentrations are the highest glucose concentrations
757 achieved known to the authors at the time of writing this review.

758 One of the highest solids loadings in enzymatic hydrolysis reported to date is 40% (w/w)
759 [29, 51]. A horizontally-oriented rotating drum was utilized as the reactor in these studies in
760 order to effectively mix the solids. The studies found that cellulose and hemicellulose
761 conversion decreased from ~90% to ~33% and ~70% to 35%, respectively, with the increase in
762 solids loading from 2% to 40%, but the reactor was providing adequate mixing as evidenced by
763 the conversion of lignocellulose into fermentable saccharides (86 g glucose/kg at 40% solids)

764 [29]. At 40% solids, liquefaction occurred after only 4 hrs. The viscosity was still high, as the
765 slurry turned into a thick, clay-like paste and remained as a thick paste following 96 hrs of
766 hydrolysis. Additionally, the reactor was a very energy efficient solution to the mixing problem.
767 Mixing speed did not affect the liquefaction time, so relatively low speeds (6.6 rpm) could be
768 used. It was also shown that ethanol could be produced in the same rotating drum reactor from
769 the resulting slurries, where the highest ethanol yield (48 g/kg DM) reported was from the slurry
770 at 35% solids. Even at reduced enzyme loadings (5 FPU/g DM supplemented with β -glucosidase
771 at a 5:1 loading), ~40% conversion for both cellulose and hemicellulose can be achieved at 30%
772 solids loading [51]. These results suggest using one reactor for all processing steps in the
773 conversion of lignocellulose, with the implication that capital and equipment costs can
774 potentially be greatly reduced as both the number of reactors and amount of enzyme used
775 decreases. However, with the yield penalty for conversion at higher solids loadings being high, a
776 full techno-economic analysis would be needed to fully validate such a system operating under
777 the given conditions.

778

779 **4.6 Tools and Methods for Measuring the Progress of Enzymatic Hydrolysis at High-Solids** 780 **Loadings**

781 As more and more interest is expressed in the use of high-solids loadings in the
782 conversion of lignocellulose, it is also important that tools are available to properly measure and
783 study the progress of the hydrolysis reaction. Calorimetry has been studied as a new tool for
784 determining enzymatic kinetics of high-solids loadings in hydrolysis [58]. It provides higher
785 sensitivity than HPLC in the early stages of the hydrolysis, making calorimetry a useful tool to
786 evaluate initial rates of hydrolysis. Avicel showed that enzyme hydrolysis slowed when enzyme

787 loading of >30 FPU/g cellulose were used. It is believed that this reduction in rate is due to the
788 lack of available binding sites on the cellulose, as illustrated by the heat-flow curves converging
789 upon a single value, regardless of the enzyme loading.

790 Lavenson et al. [57] also implemented the use of new tools to monitor liquefaction and
791 the extent of hydrolysis of cellulose. Liquefaction and the spatial homogeneity of the enzyme
792 distribution in Solka-Floc suspensions (28% w/w) were monitored with magnetic resonance
793 imaging (MRI). The MRI signal is proportional to the amount of free water in the reaction,
794 which correlates to the degree of liquefaction in the system. Additionally, a penetrometer was
795 used to monitor the mechanical strength of the suspension. Measurements were taken on two
796 hydrolysis systems, where one contained a mixed Solka-Floc and enzyme suspension and the
797 other contained a Solka-Floc suspension that received an application of enzyme but no mixing.
798 Mechanical strength of the mixed suspension decreased by 20% of the initial strength after ~30
799 hrs, as compared to ~170 hrs for the unmixed suspension. Based on the MRI results, the mixed
800 samples did not show a spatial gradient, indicating uniform liquefaction when the enzyme and
801 substrate are initially well-mixed. The unmixed samples showed a slow change in spatial
802 gradients, which were attributed to ineffective diffusion of the enzyme to the substrate. Since
803 liquefaction occurs nearly six times faster for the mixed samples, it is not surprising that higher
804 final glucose concentrations are also obtained as compared to the unmixed samples and in much
805 less time. For example, the mixed suspension reached ~75 g/L glucose in only ~120 hrs,
806 whereas the unmixed suspension produced only ~50 g/L in 300 hrs. Furthermore, adequate
807 initial mixing of the enzyme and substrate resulted in an initial rate of hydrolysis an order of
808 magnitude higher (1.8 g/L/hr as compared to 0.21 g/L/hr).

809

810 **5. Reactor Design for Enzymatic Hydrolysis at High Solids**

811 Several groups studying the use of high-solids loadings for enzymatic hydrolysis have
812 embraced a horizontal orientation of the reactor [6, 29, 62, 63]. Gravitational or free-fall mixing
813 provides many advantages over typical vertical stirred tank reactors and are used in other
814 industrial processes that require mixing highly viscous slurries, like peanut butter, ketchup and
815 concrete [62, 63]. The horizontal orientation minimizes particle settling and local accumulation
816 of reaction products within the reactor, as well as ensuring better enzyme distribution. These
817 types of reactors are also easily scalable from bench-scale to pilot-scale and larger. Power
818 requirements are lower for horizontal reactors equipped with paddles over vertical stirred tank
819 reactors that provide the same level of effective mixing [62].

820 Roche et al. [63] employed free-fall mixing in their design for bench-scale reactors for
821 enzymatic hydrolysis. Polypropylene bottles (125 mL and 250 mL) were placed on a roller
822 apparatus in a horizontal orientation. The roller apparatus and bottles were placed in an
823 incubator for temperature control during enzymatic hydrolysis. This roller-bottle system
824 produced results comparable to shake flasks when utilizing intermittent hand mixing, especially
825 following enzyme addition and prior to sampling, for up to 30% solids (data not shown). At
826 20% solids loading, these two mixing schemes resulted in 80-85% cellulose conversion. The
827 roller-bottle reactors eliminated the human component of mixing, resulting in more consistent
828 mixing and better enzyme and reaction product distribution.

829 Hydrolysis studies conducted by Dasari et al. [62] utilized a horizontal reactor of
830 intermediate capacity (8 L). The reactor was constructed from a cylinder made of Pyrex glass
831 with aluminum lids fitted over the ends. An adjustable speed, rotating shaft with rubber-tipped,
832 stainless steel blades attached was inserted into the reactor. Three sampling ports were located

833 along the length of the reactor. Hydrolysis studies comparing the horizontal reactor to shake
834 flasks found, at 25% solids loading, approximately 10% more glucose was produced in the
835 horizontal reactor.

836 Jorgensen et al. [29] developed a reactor for use in pretreatment and enzymatic hydrolysis
837 processes with a total volume of 280 L. Several features have been implemented into the pilot-
838 scale drum reactor, as well as the smaller glass reactor, to address issues associated with high-
839 solids loadings. The horizontal orientation of the reactors takes advantage of free-fall mixing,
840 eliminating the need for mechanical mixing. Evaluation of a range of mixing speeds (3.3-11.5
841 rpm) by Jorgensen et al. [29] resulted in no significant differences in cellulose conversion over
842 the tested range, so energy input for mixing is significantly reduced as compared to vertically
843 oriented stirred tank reactors. In addition to free-fall mixing, a rotating shaft affixed with
844 paddles supplies additional mixing capabilities, as the shaft in the pilot-scale reactor can be
845 programmed to change rotational direction two times per minute. The paddles also provide a
846 scraping action that removes lignocellulosic material from the reactor walls, improving heat
847 transfer between the reactor and the biomass.

848 The Integrated Biomass Utilization System (IBUS) Project coordinated by DONG
849 Energy in Denmark also utilizes free-fall reactors. DONG Energy has free-fall reactors in a
850 variety of sizes for research and development purposes (400 L) and has successfully scaled one
851 up to a capacity of 11,000 L [6, 64]. These reactors routinely operate at approximately 40%
852 solids loading. Larger particle sizes can be used, since the mechanical work of the mixing helps
853 tear biomass fibers and particles apart [6]. This tearing action also increases the surface area of
854 the lignocellulose, resulting in increased enzyme accessibility to the cellulose and hemicellulose.

855 While most reactors implemented for high-solids enzymatic hydrolysis have employed
856 some form of free-fall mixing, Zhang et al. [18] investigated the effects of a helical impeller in a
857 vertical reactor on SSF at solids loadings up to 30% (w/w) and compared it to a typical Rushton
858 (paddle) impeller (Figure 4a-b). Helical impellers are suggested for use in highly viscous, non-
859 Newtonian fluid agitation, which describes high-solids biomass slurries. The helical impeller
860 performed better than the Rushton impeller with regard to every aspect tested. The feeding rate
861 of lignocellulose into the reactor was adjusted so that a liquefied slurry could be maintained
862 throughout the feeding period. The helical impeller provided better mixing, as the feeding period
863 was completed more than 2 hr sooner than that of the Rushton impeller. The helical impeller also
864 resulted in higher ethanol concentration (51.0 g/L vs. 43.9 g/L) and productivity, as well as
865 consuming less power. At 30% solids (prior to inoculation with the fermentative organism), the
866 Rushton impeller required nearly 40 W/kg corn stover (CS) before decreasing to ~29 W/kg CS
867 after 72 hr of saccharification and fermentation. The helical impeller required ~8 W/kg CS and
868 ~1 W/kg CS prior to inoculation and after 72 hr, respectively. (It should be noted that the
869 stirring rates for the two impellers were different; however, the power requirements were
870 normalized based on the “no-load” power consumption for each impeller.) Lastly, the mixing
871 efficiency of the helical impeller was superior to the Rushton impeller. The geometry of the
872 impeller can play a significant role in effectively mixing biomass slurries. Other geometries
873 tested by Wang et al. include a plate-and-frame impeller and a double-curved-blade impeller
874 (Figure 4c-d). The impellers were tested at various speeds and 100 rpm resulted in the best
875 conversion efficiencies for both geometries. However, the plate-and-frame impeller achieved a
876 higher conversion than the double-curved-blade impeller by nearly 18%, indicating that the
877 geometry of the impeller can have an effect on the hydrolysis. The authors suggested that the

878 plate-and-frame impeller provides a more consistent mixing regime at every depth in the reactor,
879 whereas the axial flow induced by the double-curved-blade impeller is a function of the distance
880 from the blades.

881 Another study investigated the use of a peg mixer (Figure 4e) for enzymatic hydrolysis at
882 high-solids loadings [43]. The mixer used in this study was a 9 L reactor fitted with a rotating
883 shaft with pegs extending out radially. The time for liquefaction of 20% (w/w) of unbleached
884 hardwood pulp was significantly reduced when comparing shake flasks to the peg mixer (40 hr
885 vs. 1 hr). The benefit of this mixer is that it has been proven effective with lignocellulosic
886 material. High-solids enzymatic hydrolysis is just another application for the peg mixer.

887 From the various aforementioned reactors utilized with high-solids enzymatic hydrolysis
888 reactions, there are several suggestions to improve the mixing of highly viscous slurries. Free-
889 fall mixing relies on gravity to effectively mix the slurry, which consumes less energy than a
890 stirred tank reactor providing a similar degree of mixing. An effective mixing regime can greatly
891 depend on the impeller geometry, as the shape of an impeller can cause large differences in
892 speed and shear effects at various impeller-slurry interfaces throughout the reactor. High shear
893 rates have been shown to disrupt the adsorption of cellulase onto biomass or to even cause the
894 denaturation of cellulase [65, 66]. Lastly, technology should be borrowed from other
895 applications, where possible. For instance, peg mixers are a “tried-and-true” technology that is
896 commonly used in the long-established pulp and paper industry. All of these ideas have shown
897 some promise but require more study and fine-tuning before being implemented into the
898 lignocellulose conversion process.

899

900 **6. Pilot and Demonstration-Scale Operations**

901 Several plants operating at pilot- and demonstration -scale level have recently come
902 online. These installations will help the industry gain valuable insights and improve upon the
903 challenges and limitations that are not recognized at the laboratory scale.

904 One such pilot plant constructed in Denmark is operated by Inbicon (a subsidiary of
905 DONG Energy), with a distillation capacity of ~1 ton fermentation broth/hr. Additionally, in
906 2010, Inbicon opened its demonstration-scale plant that is capable of producing 5.3 million liters
907 of ethanol each year. Enzymatic hydrolysis is performed here at 25-30% (w/w) solids content
908 with a relatively low enzyme loading of 3-6 FPU/g DM. However, the plant is capable of
909 handling up to 40% (w/w) solids in any of its process streams [6, 64]. Since this operation is
910 also used for developmental purposes, they have reactors that range from 400 L up to 11,000 L.
911 Additionally, pretreatment and fermentation are performed at high-solids loadings, 20-40% and
912 ~18% DM, respectively. At the end of the conversion process, the remaining lignin-rich material
913 (40-95% DM) is burned to produce heat and electricity that can be cycled back into the
914 conversion operation.

915 The National Renewable Energy Laboratory (Golden, CO, USA) recently expanded their
916 lignocellulose processing facilities to achieve a capacity of 4,000 L and to operate at solids
917 loading of $\geq 20\%$ (w/w) [67]. The conversion process is designed as a semi-continuous operation
918 with pretreatment occurring in horizontal reactors with paddles, taking advantage of the reduced
919 energy inputs required with free-fall mixing of lignocellulose. Following liquefaction at ~24-30
920 hrs, the slurry is pumped into vertical, stirred tank reactors to complete the enzymatic hydrolysis
921 of the material. This operation is capable of processing about 0.5 to 1 ton dry biomass into
922 ethanol each day.

923

924 **7. Direction of future work**

925 In order to fully realize the benefits of operating enzymatic hydrolysis at high-solids,
926 several issues must be addressed. There are many variables associated with enzymatic
927 hydrolysis that can affect the efficiency of the conversion, including (but not limited to) biomass
928 source, pretreatment method, enzyme source and enzyme mixture. Each of these components
929 must be considered when designing a process for lignocellulose conversion, which makes
930 optimal processing conditions difficult to devise. Further study for the optimization of glucose
931 yields, especially in regards to the use of fed-batch systems, enzyme supplementation, washing
932 and detoxification steps, and additives, both individually and in combination, is still very much
933 needed. It is also important that a better understanding of some of the mechanisms that seem to
934 have the greatest impacts on the conversion process is achieved. Robust reactors capable of
935 effectively mixing biomass slurries to minimize end-product inhibition and heat and mass
936 transfer limitations are needed. Additionally, the cost of enzymes, biomass and any necessary
937 specialty equipment, as well as the best uses for any potential by-products produced in the
938 conversion process, should be considered in the design stages.

939

940 **8. Conclusions**

941 Recent national and international focus on producing biofuels and chemicals from
942 lignocellulose has led to significant research on the development and optimization of effective
943 conversion processes. Several definitive conclusions regarding enzymatic hydrolysis performed
944 at high-solids loadings can be made following a thorough review of the available literature on
945 this topic:

946

947

- Free-fall mixing is effective. The advantages of this type of mixing system are numerous, and it has been employed successfully in other industrial processes.

948

949

- The solids effect is real. Although, the exact cause of this phenomenon has not been determined, there are several hypotheses that have been suggested, including

950

951

- lower cellulase adsorption (increased concentrations of glucose and cellobiose have been shown to inhibit the adsorption of enzymes onto cellulose);

952

953

- product inhibition of enzymes occurs earlier because of the higher concentration of products;

954

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- inadequate mixing, which can emphasize diffusional limitations exacerbating product inhibition and access of enzyme to substrate;

956

957

- interaction of water with substrate (water has been shown to be more tightly bound to lignocellulose as the solids loadings increase, thus less water is available to the enzymes to perform the hydrolysis reaction).

958

959

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- Contradictory evidence continues to raise questions regarding the lignocellulose conversion process. For example, some studies have shown that washing solids following pretreatment can enhance sugar production and fermentation, while others have found the opposite to be true. Additionally, arguments persist regarding the effects water has on the overall conversion process. Lastly, as long as enzyme cost remains a large portion of the overall conversion cost, enzymes also demand further attention, especially with regards to proper loadings and combinations.

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- 967 • Fed-batch systems are worth investigating. While there have been some conflicting
968 results, many studies show overwhelming support for conducting high-solids operations
969 as a fed-batch system.
- 970 • The use of additives to reduce slurry viscosity has achieved some success at the lab-
971 scale. However, the economics of the use of additives on an industrial-scale should be
972 validated prior to implementation at that level.

973 The use of high-solids operations would make biofuels produced from the conversion of
974 lignocellulose more economical and more price-competitive with petroleum. Increasing sugar
975 and ethanol yields while reducing capital and production costs, lowering energy demands and
976 lowering water requirements will contribute to a more economically feasible process as
977 compared to one operated at low- or moderate-solids loadings. Despite all the benefits of
978 operating at high solids, the process remains restricted due primarily to the lack of available
979 water within the culture, high viscosities, which translate to difficulties with mixing and
980 handling, and increased concentration of inhibitors, which extends reaction times and increases
981 enzyme costs. Researchers are attacking these issues from many angles, experimenting with
982 different pretreatment methods and various enzyme sources and cocktails, while modifying
983 operating conditions and slurry properties. Although there has been some success at performing
984 enzymatic hydrolysis at high solids at the pilot and demonstration scale, many questions must be
985 resolved before the full potential of high-solids lignocellulose conversion will be realized.

986

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