The enzymatic hydrolysis of cellulose to glucose by cellulases is one of the major steps involved in the conversion of lignocellulosic biomass to yield biofuel. This hydrolysis by cellulases, a heterogeneous reaction, currently suffers from some major limitations, most importantly a dramatic rate slowdown at high degrees of conversion. To render the process economically viable, increases in hydrolysis rates and yields are necessary and require improvement both in enzymes (via protein engineering) and processing, i.e. optimization of reaction conditions, reactor design, enzyme and substrate cocktail compositions, enzyme recycling and recovery strategies. Advances in both areas in turn strongly depend on the progress in the accurate quantification of substrate–enzyme interactions and causes for the rate slowdown. The past five years have seen a significant increase in the number of studies on the kinetics of the enzymatic hydrolysis of cellulose. This review provides an overview of the models published thus far, classifies and tabulates these models, and presents an analysis of their basic assumptions. While the exact mechanism of cellulases on lignocellulosic biomass is not completely understood yet, models in the literature have elucidated various factors affecting the enzymatic rates and activities. Different assumptions regarding rate-limiting factors and basic substrate–enzyme interactions were employed to develop and validate these models. However, the models need to be further tested against additional experimental data to validate or disprove any underlying hypothesis. It should also provide better insight on additional parameters required in the case that more substrate and enzyme properties are to be included in a model.
which do so would be more robust, they would require more understanding of the hydrolysis process, more substrate and enzyme variables considered. Both these articles concluded that to achieve a more detailed and phenomenological potential use of various models in literature, based on the number of decreasing rates. Lee et al. (1980) in 1980 reviewed the models generated to distinguish between the hypotheses regarding the limiting causes. We also discuss the experimental data that could be associated with adsorption or surface accessibility. To modeling staged reactor configurations with different feeding frequencies of the reaction mixture. Fed-batch strategies have also been developed for the enzymatic hydrolysis of cellulose (Hodge et al., 2009). Further improvement of cellulase kinetics will be guided by the relative importance of physical parameters of the model, such as those associated with adsorption or surface accessibility. To find and alleviate bottlenecks, the kinetic and the physical parameters in the model have to be estimated correctly.

The current paper reviews the various published models of enzymatic hydrolysis of both pure cellulose and lignocellulosic materials, and gives an analysis of their key aspects as well as their shortcomings to highlight their role in advancing our understanding of this field. The experimental data present in the literature are discussed, with the aim of understanding the kinetics and rate-limiting causes. We also discuss the experimental data that could be generated to distinguish between the hypotheses regarding the decreasing rates.

Lee et al. (1980) in 1980 reviewed the models published up to that point. Zhang and Lynd (2004) discussed the potential use of various models in literature, based on the number of substrate and enzyme variables considered. Both these articles concluded that to achieve a more detailed and phenomenological understanding of the hydrolysis process, more substrate and enzyme properties have to be considered in the kinetic models. While models which did so would be more robust, they would require more experimental data for validation due to the increase in the number of variables and parameters. In any case, the two main challenges of modeling the cellulose hydrolysis process are i) to gain a more fundamental understanding of the relevant enzyme and substrate variables (substrate-concentration, degree of polymerization, accessibility, adsorption capacity, size distribution of chains, crystallinity; enzyme-concentration, cellulase composition, adsorbed cellulose concentration, synergism), and ii) to identify rate-limiting factors.

Since the last review in 2004 (Zhang and Lynd, 2004), about thirty more works have been published on kinetic modeling of cellulose bioconversion. This is more than one third of the number of works in the literature on kinetic modeling of cellulose hydrolysis by cellulases. Given the recent enthusiasm in biofuels, we believe that the time has arrived for another review on the subject.

Product inhibition of cellulases (by cellobiose) is a phenomenon that can be quantified by independent experiments and can be alleviated with an excess of β-glucosidase (Bommarius et al., 2008). The overall structure of the kinetic models of enzymatic hydrolysis of cellulose and lignocellulose is not affected by the inclusion of product inhibition parameters. The phenomenon has been previously reviewed in 2002 (Lynd et al., 2002) and 2004 (Zhang and Lynd, 2004), and the state of the art in modeling product inhibition has not advanced since then. Therefore, in this article we do not discuss the various expressions used for product inhibition. However, we also discuss the incorporation of adsorption of cellulases on cellulose substrates into the various models and the interchangeability of models for pure cellulose vs. lignocellulose substrates.

2. Model classes and classification

Biohydrolysis of cellulose, due its heterogeneous nature, involves more steps than classical enzyme kinetics. The major steps are (Fig. 1):

1. Adsorption of cellulases onto the substrate via the binding domain (Stählb erg et al., 1991).
2. Location of a bond susceptible to hydrolysis on the substrate surface (Tervu et al., 1997) (chain end if cellobiohydrolase, cleavable bond if endoglucanase).
3. Formation of enzyme–substrate complex (by translocation of the chain end into the catalytic tunnel if cellobiohydrolase, to initiate hydrolysis) (Divne et al., 1998; Mulakala and Reilly, 2005).
4. Hydrolysis of the β-glucosidic bond and subsequent forward sliding of the enzyme along the cellulose chain (Divne et al., 1998; Mulakala and Reilly, 2005).
5. Desorption of cellulases from the substrate or repetition of step 4 or steps 2/3 if only the catalytic domain detaches from chain.
6. Hydrolysis of cellobiose to glucose by β-glucosidase (if present in the enzyme mixture). In addition, product inhibition (Bezerra and Dias, 2005; Holtzapffe et al., 1990; Xiao et al., 2004; Yue et al., 2004) and changes in the substrate properties along the course of hydrolysis affect the above steps (see Section 3).

Based on the fundamental approach and methodology used, the models can broadly be divided into four classes: empirical models (Section 2.1.), Michaelis–Menten based models (Section 2.2.), models accounting for adsorption (Section 2.3.), and those models developed for soluble substrates (Section 2.4.) (see Tables 1A–D). In addition, there are two models in the literature based on jamming and fractal kinetics (discussed in Section 3.5).

2.1. Empirical models

Empirical models help in quantifying the effects of various substrate and enzyme properties on hydrolysis. Table 1A provides a list of empirical models in the literature, along with their predicted and independent variables. These empirical models have been generally used to correlate hydrolysis with either the structural properties of the substrate or with time (Table 1A). Though empirical models are not applicable outside the conditions under which they are developed and
**Table 1A**

Empirical models (BG–β-glucosidase).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Y (predicted variable)</th>
<th>X (independent variable)</th>
<th>Substrate</th>
<th>Enzyme source</th>
<th>Validation range of conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gharpuray et al. (1983)</td>
<td>Extent of hydrolysis</td>
<td>Crystallinity, lignin, specific surface area, Time</td>
<td>Pretreated winter crop wheat straw</td>
<td>T. reesei</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Ohmine et al. (1983)</td>
<td>Conversion</td>
<td>Time</td>
<td>Avicel</td>
<td>T. viride</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Sattler et al. (1989)</td>
<td>Conversion</td>
<td>Time, fractions of easily and difficult hydrolysable part</td>
<td>Pretreated poplar wood</td>
<td>Celloclast + BG (Novo, Denmark)</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Koulias et al. (1992)</td>
<td>Conversion, maximum conversion</td>
<td>Time, lignin, crystallinity</td>
<td>Ball milled Avicel, ball milled alkali-treated straw, ball milled wheat straw, alkali-treated wheat straw</td>
<td>Fusarium oxysporum</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Ooshima et al. (1991)</td>
<td>Conversion, hydrolysis rate, adsorbed enzyme conversion</td>
<td>Time</td>
<td>Avicel, pretreated Wilner hardwood</td>
<td>T. reesei, T. viride</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Kurakake et al. (1995)</td>
<td>Conversion, hydrolysis rate, adsorbed enzyme conversion</td>
<td>Time</td>
<td>NaOH pretreated pine wood</td>
<td>T. reesei + BG</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Parajó et al. (1996)</td>
<td>Conversion</td>
<td>Time, fractions of easily and difficult hydrolysable parts</td>
<td>Ball milled Avicel, filter paper, Greek purified cotton and hort-alkali-delignified wheat straw</td>
<td>Fusarium oxysporum and Neurospora crassa</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Tarantili et al. (1996)</td>
<td>Conversion</td>
<td>Maximum conversion, time for achieving half of maximum conversion</td>
<td>Enzyme to substrate ratio, liquor to solid ratio</td>
<td>Pretreated wood chips</td>
<td>Celluclast (Novo Denmark)</td>
</tr>
<tr>
<td>Môldes et al., (1999)</td>
<td>Maximum rate of cellulose conversion, max. rate of glucose generation</td>
<td>1 b and final conversions of glucan and xylan content</td>
<td>Lignin content, acetyl content, glucan content, crystallinity index</td>
<td>Hybrid poplar, bagasse and switchgrass</td>
<td>Cytolase (cellulase from Environmental BioTechnologies, Santa Rosa, CA) + BG</td>
</tr>
<tr>
<td>Chang and Holtzapple (2000)</td>
<td>Conversion</td>
<td>Initial hydrolysis rate, 72 h extent of hydrolysis Hydrolysis yields of glucan, xylan and holocellulose</td>
<td>Residual lignin</td>
<td>Corn Stover</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Park et al. (2002)</td>
<td>Conversion</td>
<td>Crystallinity, Spectroscopic features</td>
<td>Waste office paper</td>
<td>T. viride, Acremonium cellulolyticus</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Laureano-Perez et al. (2005)</td>
<td>Conversion</td>
<td>Time, enzyme concentration</td>
<td>Cellulase from NREL + BG</td>
<td>Cellulase from NREL + BG</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Kim and Holtzapple (2006)</td>
<td>Conversion</td>
<td>Crystallinity, Spectroscopic features</td>
<td>Residual lignin</td>
<td>Pretreated corn stover</td>
<td>Spizyme CP from NREL + BG</td>
</tr>
<tr>
<td>Vásquez et al. (2007)</td>
<td>Glucose concentration</td>
<td>pH, enzyme loading, temperature, solid percentage</td>
<td>Acid hydrolyzed sugarcane bagasse</td>
<td>GC 220 (Genencor International, Inc.)</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Berlin et al. (2007)</td>
<td>Glucan to glucose and xylan to xylose conversion</td>
<td>Weights of xylan, pectinase and β-glucosidase</td>
<td>Milled corn stover, dilute acid pretreated corn stover</td>
<td>Cellulase 1.5L + BG (Novozymes), xylan and pectinase (Genencor International)</td>
<td>&gt;70</td>
</tr>
<tr>
<td>O’Dwyer et al. (2008)</td>
<td>Slopes and intercepts of the graphs of 1 h, 6, 72 h glucan content vs enzyme loading</td>
<td>Crystallinity, lignin and acetyl content</td>
<td>Pretreated poplar wood</td>
<td>T. reesei</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Kim et al. (2009)</td>
<td>Reducing sugar concentration, ethanol concentration</td>
<td>pH, temperature, enzyme inoculation, reaction time</td>
<td>Food waste</td>
<td>Spirizyme Plus FG (Novozymes, Denmark)</td>
<td>–</td>
</tr>
<tr>
<td>Zhou et al. (2009)</td>
<td>Glucose produced after 72 h hydrolysis</td>
<td>Concentrations of Cel7A, Cel6A, Cel6B, Cel7B, Cel12A, Cel61A</td>
<td>Steam-exploited corn stover</td>
<td>T. viride (Cel7A, Cel6A, Cel6B, Cel7B, Cel12A, Cel61A) + BG</td>
<td>&lt;70</td>
</tr>
</tbody>
</table>

(In bold are shown the assumptions regarding the decrease in rates)

---

Fig. 1. Steps 1 to 4 for a cellobiohydrolase acting on a cellulosic substrate (not drawn to scale). For endoglucanase, steps 2 and 3 are different as it does not require chain ends to act on. Step 1—Adsorption, step 2—location of chain end, step 3—formation of enzyme–substrate complex, and step 4—hydrolysis of the β-glycosidic bond. (Note: In step 3, some authors have suggested the possibility for the cellulose chain to thread into the catalytic domain by going over the binding domain (Reinikainen et al., 1992). Insufficient experimental evidence is available yet to determine the exact mechanism).

do not provide any insight into the mechanistic details of the process, they are helpful in numerous ways:

a) They can help in understanding the interactions between the substrate properties. It has been shown that the effects of an individual substrate property such as crystallinity, lignin content, or acetyl content can depend on the levels of the other two (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006; O’Dwyer et al., 2008).

b) Empirical models can be useful for initial rate estimations, which are important for resuspension experiments (described in Sections 3.2 and 3.3) and Lineweaver–Burk plots (Linewaver and Burk, 1934) used in the Michaelis–Menten models. The rate of hydrolysis...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Substrate</th>
<th>Enzyme source (purified component if any)</th>
<th>Declining rate reason (in addition to PI)</th>
<th>Conversion range for validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang (1975)</td>
<td>Ads, QSS</td>
<td>Amorphous Solka Floc</td>
<td>T. viride</td>
<td>–</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Suga et al. (1975)</td>
<td>M-M</td>
<td>Theoretical study</td>
<td>Solka Floc</td>
<td>T. viride</td>
<td>–</td>
</tr>
<tr>
<td>Howell and Stuck (1975)</td>
<td>M-M</td>
<td>Alpha cellulose fiber</td>
<td>T. viride Cellulohydrodolase (then known as the C1 enzyme)</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Maguire (1977)</td>
<td>Ads</td>
<td>Ball milled delignified cellulose</td>
<td>T. reesei + BG</td>
<td>two phases — crystalline + amorphous</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Converse et al. (1988)</td>
<td>Ads, QSS</td>
<td>Solka Floc</td>
<td>T. reesei</td>
<td>Accessibility decreases with increase in CrI</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Howell and Lee (1983)</td>
<td>Ads</td>
<td>Solka Floc</td>
<td>T. reesei + BG</td>
<td>Decrease in Substrate reactivity</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Asojo (1982); Asojo (1984)</td>
<td>Ads</td>
<td>Solka Floc</td>
<td>T. viride</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Beltrame et al. (1984)</td>
<td>M-M</td>
<td>Textile, cotton waste, pretreated pulp</td>
<td>T. viride + BG</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Holtzapple et al. (1984)</td>
<td>Ads, QSS</td>
<td>Solka Floc</td>
<td>T. viride + BG</td>
<td>Accessibility is included as a parameter</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Scheiding et al. (1984)</td>
<td>M-M</td>
<td>Avicel</td>
<td>T. reesei + BG</td>
<td>Enzyme deactivation, amorphous + crystalline fractions</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Wald et al. (1984)</td>
<td>Ads, QSS, Apparent rate order</td>
<td>Rice straw</td>
<td>T. reesei + BG</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Caminal et al. (1985)</td>
<td>M-M</td>
<td>Microcrystalline cellulose form Merk</td>
<td>Cellulose from Merk</td>
<td>Enzyme deactivation</td>
<td>&gt;70% (only fitting)</td>
</tr>
<tr>
<td>Gusakov et al. (1985)</td>
<td>M-M</td>
<td>Chemically treated cotton stalks</td>
<td>Trichoderma longibrachiatum + BG</td>
<td>Enzyme inactivation, two phase substrate model</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Converse et al. (1988)</td>
<td>Ads, QSS, rate constant time dependent M-M</td>
<td>Microcrystalline cellulose form Merk Filter paper</td>
<td>T. viride</td>
<td>Enzyme deactivation, bulk mass transfer limitation Some fraction non-degradable</td>
<td>&gt;70% (only fitting)</td>
</tr>
<tr>
<td>Nakasaki et al. (1988)</td>
<td>Ads, accessibility characterized by surface area (scientific note) M-M</td>
<td>Pretreated wood</td>
<td>T. reesei + BG</td>
<td>Change in surface area of substrate Fitted to initial hydrolysis ate</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Philippidis et al. (1993); Philippidis et al. (1992)</td>
<td>Ads</td>
<td>Alpha cellulose, cellulbiose and gluconolactone</td>
<td>T. reesei + BG</td>
<td>Enzyme deactivation, adsorption of cellulase and β-glucosidase onto lignin, substrate reactivity coefficient included for substrate reactivity</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Converse and Optekar (1983)</td>
<td>Ads</td>
<td>Avicel</td>
<td>data from (Woodward et al., 1988) (T. reesei (Cel6A, Cel7A, Cel5A))</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Nidezisky and Steiner (1993)</td>
<td>Ads, M-M, two phase substrate</td>
<td>Sigmaell, Avicel, alpha-cellulose, cotton linens Wheat straw</td>
<td>Celluclast + BG (from Novo, Denmark) Celluclast + BG (from Novo, Denmark)</td>
<td>Enzyme desorption, two phases of substrate</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Nidezisky et al. (1993)</td>
<td>Ads, M-M</td>
<td>Whatman no. 1 Filter paper</td>
<td>T. reesei (Cel7A, Cel6A, Cel 7B) + BG</td>
<td>–</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Nidezisky et al. (1994b)</td>
<td>Ads</td>
<td>Data of Nutor and Converse (1991)</td>
<td>T. reesei</td>
<td>Decrease in substrate reactivity</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>South et al. (1995)</td>
<td>Ads</td>
<td>Pretreated corn cob</td>
<td>Trichocherum reesei/ Aspergillus niger</td>
<td>Enzyme deactivation</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Lun et al. (1997)</td>
<td>Ads</td>
<td>Theoretical study</td>
<td>Pretreated wood chips</td>
<td>Celluclast (from Novo, Denmark) Iogen super clean cellulase</td>
<td>–</td>
</tr>
<tr>
<td>Fenske et al. (1999)</td>
<td>Ads, shrinking particle theory</td>
<td>Microcrystalline cellulose from Merck Alpha cellulose, ball milled and untreated steam exploded wood</td>
<td>Celluclast + BG (from Novo, Denmark) Celluclast + BG (from Novo, Denmark)</td>
<td>Inactive complexes formed on substrate Substrate reactivity, enzyme deactivation</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Moldes et al. (1999)</td>
<td>Ads</td>
<td>Data from Stenberg et al. (2000) (Steam-pretreated softwood)</td>
<td>Celluclast 2L + BG (from Novo, Denmark)</td>
<td>Decrease in cellulose specific surface area, adsorption of cellulase and β-glucosidase onto lignin Inert fraction of cellulase, enzyme deactivation</td>
<td>&lt;70%</td>
</tr>
</tbody>
</table>

Table 1B (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Substrate</th>
<th>Enzyme source (purified component if any)</th>
<th>Declining rate reason (in addition to PI)</th>
<th>Conversion range for validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movagharnejad (2005); Movagharnejad and Sohrabi (2003)</td>
<td>Ads, shrinking particle theory</td>
<td>Cellulosic waste materials</td>
<td>Cellulase + BG (from Novo, Denmark)</td>
<td>Inaccessibility of active sites to enzyme</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Bezerra and Dias (2004)</td>
<td>Evaluation of M-M models</td>
<td>Avicel</td>
<td>T. reesei (Cel7A)</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Shen et al. (2004)</td>
<td>Ads</td>
<td>Dried cotton, viscose and flax yarns</td>
<td>Trichoderma pseudokoningii</td>
<td>–</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Ding and Xu (2004)</td>
<td>Ads, Q5S</td>
<td>PASC, Avicel, PCS</td>
<td>T. reesei (Cel7A and Cel7B) and H insolens (Cel6A and Cel7A)</td>
<td>–</td>
<td>Evaluation of accessibility with initial rates only</td>
</tr>
<tr>
<td>Kadam et al. (2004)</td>
<td>Ads</td>
<td>Pretreated corn stover</td>
<td>CPN commercial cellulase (logen Corp.) + BG</td>
<td>Substrate reactivity</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Lin et al. (2005)</td>
<td>Ads</td>
<td>Cellulose powder 101-F (Sigma, USA)</td>
<td>T. reesei + BG</td>
<td>Adsorbed enzyme converted irreversibly into inactive complex</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Shin et al. (2006)</td>
<td>M-M</td>
<td>Alpha cellulose, ball milled and untreated steam exploded wood</td>
<td>Data from (Moon et al., 2001) (Cellulase + BG from Novo, Denmark)</td>
<td>Inhibition by lignin, enzyme deactivation</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Ljunggren (2005)</td>
<td>Ads</td>
<td>Pretreated spruce, pretreated sugar cane bagasse</td>
<td>Cellulase + BG (from Novo, Denmark)</td>
<td>Enzyme deactivation, β-glucosidase adsorption to lignin</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Zhang and Lynd (2006)</td>
<td>Ads</td>
<td>PASC, Avicel, bacterial cellulose, cotton, filter paper</td>
<td>Results compared with literature on T. reesei cellulase system (Cel7A, Cel6B, Cel7B)</td>
<td>–</td>
<td>Parameter values taken from literature, initial rates compared with data from Wood (1975)</td>
</tr>
<tr>
<td>Drissen et al. (2007)</td>
<td>M-M, Ads</td>
<td>Avicel, Whatman filter paper, wheat straw</td>
<td>Cellubrix (Novozymes Corp., Denmark) + BG</td>
<td>Enzyme deactivation, decreasing reactivity of substrate</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Peri et al. (2007)</td>
<td>Ads</td>
<td>Non crystalline cellulose (prepared from cotton and α-cellulose), α-cellulose</td>
<td>Spezyme CP (Genencor)</td>
<td>–</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>O’Dwyer et al. (2007)</td>
<td>Same model as Holtzapple et al. (1984)</td>
<td>Lime-pretreated corn stover</td>
<td>T. reesei + BG</td>
<td>–</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Al-Zuhair (2008)</td>
<td>Ads</td>
<td>Highly crystalline wood shavings, carboxymethylcellulose (CMC)</td>
<td>Aspergillus niger</td>
<td>Two phase substrate</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Liao et al. (2008)</td>
<td>Ads</td>
<td>Lignocellulosic material from dairy manure</td>
<td>Cellulase + BG (from Sigma)</td>
<td>Decreasing substrate reactivity</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Shen and Agblevor (2008a)</td>
<td>Ads, Q5S</td>
<td>Steam-exploded cotton gin waste</td>
<td>Novozymes NS 50052 (from Novozymes) and Spezyme A03117 (from Genencor International)</td>
<td>Enzyme deactivation</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Shen and Agblevor (2008b)</td>
<td>Ads, Q5S</td>
<td>Cotton gin waste, recycled paper sludge</td>
<td>Spezyme A03117 (Genencor International)</td>
<td>Enzyme deactivation</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>Shao et al. (2009a)</td>
<td>Ads</td>
<td>Waste paper sludge</td>
<td>Spezyme CP (Genencor) + BG (Sigma-Aldrich)</td>
<td>Decreasing substrate reactivity</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Zheng et al. (2009b)</td>
<td>Ads</td>
<td>Creeping wild ryegrass</td>
<td>Cellulase + BG (Novozymes Inc.)</td>
<td>Decreasing substrate reactivity, adsorption to lignin</td>
<td>&lt;70%</td>
</tr>
</tbody>
</table>

For enzymatic hydrolysis of cellulose, to avoid the effects of product inhibition at product concentrations equal to zero, initial rates are plotted on the y axis vs. the reciprocal of the substrate concentration (in the Lineweaver–Burk plot) (Beltrame et al., 1984; Gusakov et al., 1985; Huang, 1975; Maguire, 1977; Ryu et al., 1982; Shen and Agblevor, 2008a). These initial rates can be estimated using empirical expressions, such as:

i) Differentiating expressions by Sattler et al. (1989) (Eq. (2)) and Koullas et al. (1992) (Eq. (4)) with respect to time to get Eqs. (3) and (5):

Sattler et al. (1989):

\[ Y = (C_a + C_b) - C_a e^{-k_1t} - C_b e^{-k_2t} \]  

(2)
d) Spectroscopic data — seven main peaks of the DRIFT spectra (Diffusive Reflectance FT-IR) of the substrate, along with crystallinity and lignin content have also been used as an independent variable (model inputs) in statistical models to predict the initial hydrolysis rate (3 hour glucan yield) and 72 hour hydrolysis extent (Laureano-Perez et al., 2005). The various levels of these factors were achieved by pretreating the substrate to different extents. Once the desired levels of the model inputs are determined, pretreatment conditions can be set to achieve the values closest to the optimal ones.

2.2. Michaelis–Menten based models

The Michaelis–Menten scheme (Michaelis and Menten, 1913) is based on mass action laws that hold for homogenous reaction conditions and hence cannot be directly applied to the heterogeneous reaction conditions of enzymatic hydrolysis of insoluble cellulosic substrates. The excess substrate to enzyme ratio condition ([S] ≫ [E]), which is usually employed for the quasi-steady state assumption (Laidler, 1955; Schnell, 2003), is not achieved since the fraction of cellulose accessible for adsorption ranges from 0.002 to 0.04 (Hong et al., 2007). The excess substrate condition, even if ever achieved initially, could not be retained at higher conversions as the substrate gets depleted. It has also been pointed out by Lynd et al. (2002) that the concentration of adsorbed cellulase depends on the substrate concentration and that dual saturation is possible by keeping the enzyme or substrate concentration high; these features are not characteristic of Michaelis–Menten kinetics. Cellulose hydrolysis is a heterogeneous reaction occurring on the substrate surface and is therefore a reaction occurring in dimensions less than three. For heterogeneous reaction systems, classical chemical kinetics assumption of uniformly mixed systems does not hold, resulting in apparent rate orders, time-dependent rate constants, and non-uniform concentration variation of reacting species in the fractal or dimensionally restricted media (Anacker and Kopelman, 1987; Kopelman, 1986; Kopelman, 1988). Such a behavior is termed fractal kinetics. Monte Carlo simulations have corroborated that the quasi-steady state assumption cannot be applied in these reaction systems (Berry, 2002). Conversion of cellulbiose to glucose by β-glucosidase, however, can be modeled by Michaelis–Menten kinetics since it is a homogeneous reaction.

However, Michaelis–Menten models in the literature fit the experimental data very well under the conditions they were developed. Bezerra and Dias (2004) have tested eight different Michaelis–Menten models against data of Avicel hydrolysis by T. reesei Cel7A for 24 different substrate-to-enzyme ratios. A model with competitive inhibition by cellulbiose was found to fit the data best. Reasons for the decreasing rates such as nonproductive cellulase binding, parabolic inhibition, and enzyme deactivation were shown to be insignificant in comparison to substrate depletion and competitive inhibition. Another work on Avicel with a fungal cellulase system from T. viride (Ohmine et al., 1983), however, had shown earlier that the same Michaelis–Menten model, incorporated with changes due to crystallinity and enzyme deactivation too, over-predicted the hydrolysis data. It was therefore suggested that either the kinetic scheme of the reaction is completely different or rate-retarding factors related to substrate heterogeneity are involved. The substrate heterogeneity factors are analyzed in Section 3 (‘Rate limitations and decreasing rates with increasing conversion’).

### Table 1C

Models on soluble cello-oligosaccharides (DP – Degree of polymerization).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Substrates</th>
<th>Enzyme source (pure component if any)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujii and Shimizu (1986)</td>
<td>Carboxy methyl cellulose and hydroxyl ethyl cellulose</td>
<td>Trichoderma koningii</td>
</tr>
<tr>
<td>Schmid and Wandrey (1989)</td>
<td>Cellooligosaccharides with chain lengths of 2</td>
<td>β-glucosidase from T. reesei</td>
</tr>
<tr>
<td>Nassar et al. (1991)</td>
<td>Model validated with data from Schmid and Wandrey</td>
<td></td>
</tr>
<tr>
<td>Dean and Rollings (1992)</td>
<td>Dextran (poly saccharide with α-1,6-glycosidic linkages)</td>
<td>Endoexoglucanase from Arthrobacter globiformis</td>
</tr>
</tbody>
</table>

### Table 1D

Models on jamming and fractal kinetics.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Substrate</th>
<th>Enzyme source (pure component if any)</th>
<th>Range of validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valjama et al. (2003)</td>
<td>Bacterial cellulose T. reesei (Cel7A, Cel5A)</td>
<td>(~10%) (Note: The objective was to fit the data to find the parameter h representing the fractal dimension)</td>
<td></td>
</tr>
<tr>
<td>Xu and Ding (2007)</td>
<td>Avicel H. insolens (Cel7A), and PASC</td>
<td>T. reesei (Cel7A)</td>
<td>(~70%)</td>
</tr>
</tbody>
</table>

\[
\frac{dY}{dt} \bigg|_{t=0} = C_kk_0 + C_hk_h
\]

where \( Y \) is the concentration of hydrolyzed cellulose, \( C_k \) and \( C_h \) are concentrations of easily and difficult hydrolysable parts of cellulose respectively, \( k_0 \) and \( k_h \) are the rate constants of the first order hydrolysis of easily and difficult hydrolysable parts of cellulose, \( t \) is time, \( dt/dt (t=0) \) is the initial rate.

Koullas et al. (1992):

\[
x = x_{\text{max}} \frac{t}{t_{1/2} + t}
\]

where \( x \) is the conversion of cellulose to glucose, \( x_{\text{max}} \) is the maximum conversion, \( t_{1/2} \) is the time required for 50% conversion, \( t \) is time, \( dx/dt (t=0) \) is the initial rate.

ii) Estimating \( v_p \) in the expression by Ohmine et al. (1983) (see above, Eq. (1)).

c) When large data sets are available, statistical models can be used to optimize reaction conditions (Kim et al., 2008; Vásquez et al., 2007). Two examples employed response surface methodology to find optimal levels (to maximize cellulose conversion to glucose) of the operating conditions (pH, temperature, enzyme loading and solid percentage by Vásquez et al. (2007), pH, temperature and enzyme concentration by Kim et al. (2008)). Response surface methodology has also been used for optimizing cellulase mixtures (Berlin et al., 2007; Zhou et al. 2009). Using steam-exploded corn stover as the substrate, Zhou et al. (2009) optimized the composition of a mixture of T. viride cellulases (Cel7A, Cel6A, Cel6B, Cel7B, Cel12A, Cel61A and β-glucosidase) to maximize glucose production. O’Dwyer et al. (2008) developed a neural network model to predict conversion levels as a function of crystallinity index, lignin content and acetyl content using data from 147 poplar wood samples. Such models which interpolate over a large range of the predicted and independent variables can be considered to have robust parameter values and can be useful for designing processes under various conditions.
2.3. Adsorption in cellulose hydrolysis models

Incorporation of adsorbed cellulase concentration into hydrolysis models has been achieved mainly in two ways: with the Langmuir adsorption isotherm, or with the help of kinetic equations. Fan and Lee (1983) observed constant amount of adsorbed cellulase per weight of cellulose along the hydrolysis and so a constant specific adsorption amount was used in their analysis. Movagharnejad et al. (2000) modeled the available number of active sites on the substrate surface as proportional to the surface area of the cellulose particles.

An example of a model employing the Langmuir adsorption isotherm is the one by Kadam et al. (2004). The adsorbed amount is given by:

\[ E_{\text{ads}} = \frac{E_{\text{max}} K_{\text{ad}} E_f S}{1 + K_{\text{ad}} E_f} \]  

where \( E_b \) is the bound enzyme concentration, \( E_f \) is the free enzyme concentration, \( K_{\text{ad}} \) is the dissociation constant for adsorption, \( S \) is the substrate concentration, and \( E_{\text{max}} \) is the maximum adsorption capacity in amount of cellulase per amount of cellulose.

An example of the models using kinetic equations for the amount of enzyme adsorbed is the one by Gan et al. (2003) where the following equations were used for the adsorbed species:

\[ E + S_c k_{\text{sc}1} E^*S_c \]

\[ \frac{dC_{\text{E}}}{dt} = k_{\text{ad}} C_{\text{E}} C_{\text{S}} - k_{\text{sc}2} C_{\text{E}}^*S_c - k_p C_{\text{E}}^*S_c \]

where \( E \) is the enzyme, \( S_c \) is the active cellulose, \( E^*S_c \) is the enzyme–cellulose complex, \( C_{\text{E}} \) is the enzyme concentration, \( C_{\text{E}}^*S_c \) is the enzyme–cellulose complex concentration, \( C_{\text{sc}} \) is the active cellulose concentration, \( k_{\text{ad}} \) is the adsorption constant on active cellulose, \( k_{\text{sc}2} \) is the desorption constant on active cellulose, and \( k_p \) is the product formation constant.

Some of the models (Al-Zuhair, 2008; Brown and Holtzapple, 1990; Converse et al., 1988; Drissen et al., 2007; Fan and Lee, 1983; Gan et al., 2003; Huang, 1975; Kadam et al., 2004; Lin et al., 2005; Moon et al., 2001; Nidetzky and Steiner, 1993; Peri et al., 2007; Shen and Agblevor, 2008a; South et al., 1995; Wald et al., 1984) assume instantaneous substrate–enzyme complex formation (fully productive adsorption), so the adsorbed amount of cellulase is the same as the amount of substrate–enzyme complexes. Some others (Asenjo, 1984; Converse and Optekar, 1993; Ding and Xu, 2004; Holtzapple et al., 1984; Liao et al., 2008; Luo et al., 1997; Ryu et al., 1982) assume an additional kinetic step on the substrate surface after cellulase adsorption, as did Luo et al. (1997), where the adsorbed cellulase combines with substrate to form a cellulase–substrate complex:

\[ E' + C \xrightarrow{K_1} E'C \]

where \( E' \) is the adsorbed enzyme on the active sites, \( C \) is cellulose, \( K_1 \) is the equilibrium constant, and \( E'C \) is the cellulase–substrate complex. Brown and Holtzapple (1990) and Holtzapple et al. (1984) used the quasi-steady state assumption for the adsorbed enzyme and the substrate–enzyme complex species.

While isotherms other than the Langmuir isotherm, such as the Langmuir–Freundlich isotherm (Medve et al., 1997) and two-site models (Medve et al., 1998; Medve et al., 1997; Ståhlberg et al., 1991), have been shown to fit the data, only the Langmuir isotherm has been used in hydrolysis models. However, the Langmuir isotherm should only be used as a mathematical expression since its underlying assumptions (reversibility, non-interacting adsorbed species, homogenous binding sites and uniform composition of adsorbed cellulase mixture) may not be valid in all situations (Zhang and Lynd, 2004).

While using the Langmuir isotherm or any other mathematical expression for calculating the adsorbed amount of enzyme during hydrolysis, an implicit assumption is that the adsorption equilibrium is established very fast as compared to the hydrolysis step. According to Steiner et al. (1988), this assumption may not be valid under all experimental conditions. The time to reach equilibrium adsorption has been estimated to be of the order of 5–60 min (Bader et al., 1992; Beldman et al., 1987; Ghose and Bisaria, 1979; Kim et al., 1994; Medve et al., 1998; Medve et al., 1994; Nidetzky et al., 1994a; Ståhlberg et al., 1991; Steiner et al., 1988). Though the time required for complete hydrolysis of cellulose (100% conversion) is usually 25–100 h (Bommarius et al., 2008; Bertran and Dale, 1985; Gregg and Saddler, 1996; Nutor and Converse, 1991; Tu et al., 2007), the time for low conversion levels is two to three orders of magnitude lower (Bommarius et al., 2008; Hong et al., 2007; Nutor and Converse, 1991; Väljamäe et al., 1998). Also, use of the same isotherm at all time points of the reactions assumes that adsorption characteristics of the substrate–enzyme system do not change. If both assumptions (equilibrium of the adsorption and a single isotherm valid for all conversion levels) hold true, then the amount of enzyme adsorbed per unit weight of the substrate can only increase (see below).
However, it is seen that $E_{\text{ads}}$ does not monotonically increase with conversion, for both pure cellulosic substrates (Fan and Lee, 1983; Huang, 1975; Jeoh et al., 2006; Kurakake et al., 1995; Nidetzky and Steiner, 1993; Steiner et al., 1988) and lignocellulosic substrates (Kurakake et al., 1995; Liao et al., 2008; Nutor and Converse, 1991; Shen and Agblevor, 2008a; Steiner et al., 1988). Hong et al. (2007), working with Avicel, have shown that the maximum adsorbable amount ($E_{\text{ads max}}$ in the Langmuir isotherm) decreases with conversion. Empirical equations have also been developed for the changing concentration of adsorbed enzyme during the hydrolysis reaction (Kurakake et al., 1995). Lignin and hemicellulose act as barriers to concentration of adsorbed enzyme during the hydrolysis reaction. Using paper sludge as the substrate, Shao et al. (2009a) modeled adsorption of cellulases by the rate Eqs. (13) and (14), and found that the same adsorption parameters fitted the data till 65% conversion; whereas, Liao et al. (2008), who used lignocellulosic material from dairy manure as the substrate, represented the change in the adsorption constant by an empirical expression (in time) fitted to the experimental data of adsorbed cellulase (Eq. (15)).

$$r_{\text{CE}} = k_{\text{f}}[E_i](1 + \alpha C) - \frac{k_{\text{b}}}{K_{\text{CE}}}[E_i]$$

(13)

$$r_{\text{LE}} = k_{\text{f}}[E_i](1 + \alpha L) - \frac{k_{\text{b}}}{K_{\text{LE}}}[E_i]$$

(14)

where CE denotes cellulase enzyme complex, LE denotes lignin enzyme complex, $r_{\text{CE}}$ and $r_{\text{LE}}$ denote the rate of formation of cellulose enzyme complex and lignin enzyme complex respectively, $s_c$ and $s_l$ denote the adsorption capacities of cellulose and lignin respectively, $k_c$ and $k_l$ are the dynamic adsorption constants, $[E_i]$, $[C]$ and $[L]$ are concentrations of free enzyme, cellulose and lignin respectively, $K_c$ and $K_l$ are the adsorption constants.

$$K = \frac{a t}{b + t}$$

(15)

where $a$ and $b$ are empirical constants, $t$ is time, and $K$ is the adsorption constant.

Nidetzky and Steiner (1993), who used four different cellulosic substrates (Sigmacell, Avicel, alpha-cellulose, cotton liners), represented the adsorption–desorption process over the conversion range as three phases: phase 1 where cellulases are adsorbed rapidly, phase 2 where desorption is linearly proportional to substrate conversion, and phase 3 where desorption occurs at a very low rate. The three works mentioned here used different substrates and the validation of the adsorption model was done independent of the kinetic model, so that the differences in the adsorption model fitting cannot be attributed to the different natures of the overall kinetic models. Cellulases were the only enzymes used in these works, so the differences in adsorption characteristics cannot be expected to be due to enzymes but are mainly due to the different nature of the substrates. The adsorption characteristics can thus be substrate-dependent.

2.4. Models on soluble cello-oligosaccharides

Only a few models have been published on the cellulase hydrolysis of soluble cello-oligosaccharides (Table 1C). Harjumäki et al. (1996) developed a kinetic model for the hydrolysis of soluble cello-oligosaccharides (with a degree of polymerization (DP) of 4–6) by Cel6A from T. reesei. When cleavage patterns were revealed, cellobiose was found to react the fastest and to inactivate Cel6A irreversibly. Similar work earlier by Nidetzky et al. (1994c) also revealed cleavage patterns by Cel7A and Cel6A from T. reesei. Binding constants increased up to a DP of 6 and then remained constant for DP of 7 and 8, providing information about the span of the active site. Nassar et al. (1991) used a stochastic model to fit the data of Schmid and Wandrey (1989), and it was found that $\beta$-glucosidase from T. reesei degrades cellohexaose (starting from length 6-cellohexaose) with the same rate down till a length 2 (cellobiose), and rate of cellohexaose degradation was estimated to be much smaller. Using soluble cellulose derivative substrates, carboxymethyl cellulose and hydroxyethyl cellulose, Fuji and Shimizu (1986) modeled synergism using the model developed by Fujii et al. (1981) which was based on the Michaelis–Menten scheme. The synergistic effect of endo-enzymes on the exo-enzymes (resulting from random cleavages giving rise to more cellulose chains for exo-enzymes to act on) was found to exist until the molecular weight of the substrate decreased to 4000.

While the above-mentioned models can be used to describe the hydrolysis of soluble substrates, extension to insoluble substrates is not straightforward. This is mainly because of the heterogeneous nature of cellulase action on insoluble cellulosic substrates. The concentration and distribution of accessible chain ends in insoluble substrates is also not known. However, once the issue of accessibility of chain ends is solved, cellulase hydrolysis can be modeled as polymer degradation by enzymes as was achieved by Okazaki and Moo-Young (1978) (as an example only one of the equations developed in that work is shown):

$$\frac{d[C_i]}{d\tau} = \left(\frac{K_{\text{MT}}}{K_{\text{MT}} + \sum_{i=3}^{\infty} \left(C_i - (i-1)!|C_i|\right)}\right)^{-1} \frac{1}{K_{\text{C}} + [C_2] / K_{\text{C}} + [C_1] / K_{\text{C}} + [C_3] / K_{\text{C}}}$$

(16)

where $[C_i]$ is the concentration of cellulose with chain length $i$, $[C_1]$ and $[C_2]$ are concentrations of glucose and cellobiose respectively, $K_c$ is the reaction rate constant, $[E_i]$ is the concentration of endoglucanase, $K_{\text{MT}}$ is the Michaelis constant, $K_{\text{C}}$, and $K_{\text{C}}$ are the inhibition constants of $E_i$ by glucose and cellobiose respectively. $C_1$ is degraded by exoglucanase also and a similar expression as the one on the right hand side can be written for it and added to the rate.

With a recent study claiming that cellulase hydrolysis leads to the production of cello-oligosaccharides that are possibly not degraded by endoglucanases and exoglucanases (Gupta and Lee, 2008), models on soluble cellulosic substrates might provide more insight into the hydrolysis mechanism.

Recently, Ting et al. (2009) developed a stochastic model which gave insights into the modularity of the cellulases. The catalytic domain (CD) and the cellulose binding domain (CBD) were modeled as random walkers whose dynamics were coupled by the compression/expansion of the linker and lifting of cellulase chain from the substrate surface. For simplicity, only the major governing equation is shown:

$$\frac{dP(x, r, t)}{dt} = k_c(r + 1)P(x = 0, r + 1, t) + k_{\text{b}}(r + 1)P(x, r, 1, t) + k_{\text{b}}(r - 1)P(x, r - 1, t) - [k_c(r) + K_{\text{b}}(r) + k_{\text{b}}(r)]P(x, r, t)$$

(17)

where $x$ denotes the position of CD, $r$ is the separation between the CD and CBD, $P$ denotes the probability of CD being at position $x$ (the first
entry in the parenthesis) with separation $r$ (second entry in the parenthesis) from the CBD at time $t$ (third entry in the parenthesis), $k_C(r)$ is the transition probability per unit time (for the CD) to move towards the CBD to a distance of $r-1$ from $r$, $k_{C-}(r)$ is the probability of the CBD to move away from the CD to a distance of $r+1$ from $r$, $k_{B-}(r)$ is the probability of the CBD to move towards the CD to a distance of $r-1$ from $r$.

The constants in the equations are then described by the energy dynamics arising from the compression/expansion of the linker, energy dynamics of hydrolysis, and chain disruption from the crystalline substrate surface. It was found that the linker flexibility/stiffness was an important factor governing the hydrolysis rates, as was the intrinsic hydrolytic activity of the CD. This is the first kinetic model which has attempted to explain the dynamics of the cellulose hydrolysis process by capturing the modular nature of the cellulases.

### 3. Rate limitations and decreasing rates with increasing conversion

Most of the experimental studies showed that the rate of hydrolysis drops by two to three orders of magnitude at high degrees of conversions (Fig. 2, from Bommarius et al. (2008)). Even after alleviating product inhibition from cellobiose, cellulase activities and hydrolysis rates fall precipitously as the reaction proceeds (Bommarius et al., 2008). To be able to increase the rates, the various bottlenecks in cellulose hydrolysis need to be elucidated.

The contributing factors to decreasing rates (other than product inhibition) accounted for in the existing models include (see Tables 1A–D): a) enzyme deactivation (Section 3.1), b) biphasic composition of cellulose (Section 3.2), c) decrease in substrate reactivity (Section 3.3), d) decrease in substrate accessibility (Section 3.4), e) jamming and fractal kinetics (Section 3.5), and f) decrease in the synergism between cellulases (Section 4). In this section (Section 3), we discuss these factors used in the models for both pure cellulosic and lignocellulosic substrates. For substrates containing lignin and other non-cellulosic components, additional factors such as inaccessibility caused by lignin and adsorption of cellulases to lignin will contribute to rate limitations; these aspects are discussed in Section 5.

#### 3.1. Enzyme deactivation

While enzyme deactivation has often been modeled as a first order process with respect to the total enzyme concentration (Caminal et al., 1985; Drissen et al., 2007; Ljunggren, 2005; Luo et al., 1997; Moon et al., 2001; Oh et al., 2000; Philippidis et al., 1993; Philippidis et al., 1992; Schell et al., 1999; Shin et al., 2006), inactivation of the adsorbed enzyme only has also been considered (Converse et al., 1988; Gusakov et al., 1985; Howell, 1978; Lin et al., 2005; Scheiding et al., 1984). Gan et al. (2003) considered the loss of enzyme due to shear force. Shen and Agblevor (2008a), and Shen and Agblevor (2008b) assumed enzyme deactivation to be a second-order reaction.

As an example of the enzyme deactivation of the adsorbed enzyme, Converse et al. (1988) used the following reaction representing enzyme deactivation:

$$E_a \frac{k_1}{k_2} E_d$$

where $E_a$ is the actively adsorbed enzyme, $E_d$ is the inactively adsorbed enzyme, and $k_1$ is the inactivation rate constant, $k_2$ is the reactivation rate constant.

Enzyme deactivation has also been related to enzyme clogging in an erosion model (Våljamäe et al., 1998), where the cellobiohydrolyases become stuck on the substrate surface when surrounding cellulose chains prevent further processive action. Through restart hydrolysis experiments, Yang et al. (2006) also suggested stopping or slowdown of the enzymes on the substrate surface to account for the reaction rate slowdown. Eriksson et al. (2002) showed that thermal enzyme instability and product inhibition are not the major causes for the reduction in rates. The authors proposed a model where cellobiohydrolyases encounter obstacles during their processive action while endoglucanases partially remove this hindrance by hydrolyzing the responsible cellulose chains. This study however, was performed with steam-pretreated spruce, a lignocellulosic substrate where non-cellulosic parts can also possibly act as obstacles to enzymes.

#### 3.2. Two-phase substrate

Under the assumption of a two-phase substrate, the more reactive part reacts faster resulting in a decrease in its overall fraction and a concomitant decrease in the overall reaction rate with time. Some works suggested that the amorphous part of cellulose reacts first (accompanied by an increase in crystallinity) (Chen et al., 2007; Gan et al., 2003; Lee and Fan, 1983; Mansfield and Meder, 2003; Medve et al., 1994; Ohmine et al., 1983; Ooshima et al., 1983; Szijártó et al., 2008; Våljamäe et al., 1999; Zhang et al., 1999), while constant (Lenz et al., 1990; Puls and Wood, 1991) and decreasing crystallinity (Mansfield and Meder, 2003) along conversion have also been reported. Zhang and Lynd (2004), and Mansfield et al. (1999) reported this dichotomy as well. Models assuming cellulose to be divided into crystalline and amorphous fractions have been proposed (Gusakov et al., 1985; Peiterson and Ross, 1979; Ryu et al., 1982; Scheiding et al., 1984). These works, however, did not verify their assumptions by measuring

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**Fig. 2.** Conversion-time behavior of non-pretreated Avicel at optimal ratio of activities of β-glucosidase/cellulase 1:20 (Bommarius et al., 2008); $T = 50^\circ\text{C}$, pH 5.0; $V = 8$ mL. (●) 1.5 U Cellulase (■) 15 U Cellulase (▲) 30 U Cellulase. I, II and III denote the three kinetic phases identified.
the crystallinity of the substrate along conversion. Based on a Michaelis–Menten scheme of the hydrolysis of amorphous and crystalline fractions, Ryu et al. (1982) obtained the following two equations:

$$\frac{v_{\text{max}}}{K_m} = \left( \frac{v_{\text{max},c}}{K_{M,c}} - \frac{v_{\text{max},a}}{K_{M,a}} \right) \phi + \frac{v_{\text{max},a}}{K_{M,a}}$$  \hspace{1cm} (19)

$$\frac{1}{K_m} = \left( \frac{1}{K_{M,c}} - \frac{1}{K_{M,a}} \right) \phi + \frac{1}{K_{M,a}}$$  \hspace{1cm} (20)

where \( v_{\text{max}} \) is the maximum apparent rate, \( v_{\text{max},c} \) is the maximum rate for crystalline fraction, \( v_{\text{max},a} \) is the maximum rate for amorphous fraction, \( K_{M,c} \) is the apparent Michaelis constant, \( K_{M,a} \) is the Michaelis constant for crystalline fraction, and \( \phi \) is the fraction of crystalline phase.

The two-phase hypothesis, however, was emphasized to be a simplification of the true physical complexity of cellulose. Cellulose crystallinity was shown to affect the digestibility of cellulose by simplifying the process. Nakasaki et al. (1988) assumed the non-degradable cellulose to be present in the substrate. Zuhair (2008) also used this assumption in modeling SSF with staged reactors and intermediate feeding of enzyme and substrate (Shao et al., 2009a; Shao et al., 2009b). Based on the assumption that the initial rates (for pretreated corn stover) followed a linear trend with the substrate concentration, Kadam et al. (2004) fitted the following equation for substrate reactivity:

$$R_s = \frac{S}{S_0}$$  \hspace{1cm} (23)

where \( R_s \) is substrate reactivity, \( S \) is substrate concentration, \( S_0 \) is initial substrate concentration.

Various studies have used resuspension experiments to study the reactivity of partially converted cellulose (Desai and Converse, 1997; Drissen et al., 2007; Drucek et al., 1985; Hong et al., 2007; Lee and Fan, 1983; Ooshima et al., 1991; Váljamäe et al., 1998; Yang et al., 2006; Zhang et al., 1999). As an example, Lee and Fan (1983) developed the following expression:

$$\phi = 1 - X^n$$  \hspace{1cm} (21)

where \( \phi \) is relative digestibility, \( X \) is conversion, and \( n \) is a parameter fitted with the help of resuspension experiments.

South et al. (1995) also expressed the reaction rate constant in terms of conversion:

$$k(x) = k(1-x)^n + c$$  \hspace{1cm} (22)

where \( k \) is the reaction rate constant for hydrolysis, \( x \) is conversion, \( k(x) \) is the reaction rate constant at conversion \( x \), \( n \) is the exponent of declining rate constant, and \( c \) is a constant. \( n \) and \( c \) were estimated by approximating \( k(x) \) by the ratio of rate/adsorbed enzyme and fitting it with equation to conversion \( x \). This expression was later used in modeling SSF with staged reactors and intermediate feeding of enzyme and substrate (Shao et al., 2009a; Shao et al., 2009b).

The inclusion of the rate constant or substrate reactivity as a function of conversion may fit the data well, a physical interpretation of the constants in these equations is not possible. The continuous decline in reactivity has been alternatively explained by the consumption of a more reactive fraction of the substrate (Hong et al., 2007), leading back to the assumption of a biphasic substrate.

3.3. Substrate reactivity

The change in substrate reactivity has been included in a number of models to explain the reduced digestibility of hydrolyzed cellulose, for both lignocellulosic and pure cellulose substrates (Table 1B). Some of these works will be discussed here. Lee and Fan (1983) (pure cellulosic substrate) and Moon et al. (2001) (both pure cellulosic and lignocellulosic substrates) employed the initial hydrolysis rates from resuspension experiments of spent substrate to correlate ‘relative digestibility’ with conversion. As an example, Lee and Fan (1983)
Lynd, 2004):  

$$F_a = 2\alpha A_{\text{max}} \text{MW}_{\text{anhydroglucose}}$$

(25)

where $F_a$ is the fraction of the $\beta$-glycosidic bonds accessible to cellulase, $\alpha$ is the number of cellobiose lattice occupied by the cellulase, $A_{\text{max}}$ is the maximum adsorption concentration of cellulase, and $\text{MW}_{\text{anhydroglucose}}$ is the molecular weight of anhydroglucose.

This fraction fell by approximately 50% from 0.002 until a conversion of around 85% (conversion of Avicel with T. reesei cellulase system) (Hog et al., 2007). In light of these findings, it might be important to take into account the reduced accessibility and adsorption capacity of the substrate as the conversion proceeds (also discussed in Section 2). Ding and Xu (2004) have estimated the ‘kinetic accessibility’ of Avicel and PASC to T. reesei and H. insolens cellulases from initial rate data (Eqs. (26) and (27)).

$$\phi = \frac{S_{b0}}{V_0}$$

(26)

where $[S_{b0}]$ is the concentration of cellulose available to cellulases for productive adsorption, $[S_r]$ is the total concentration of cellulose, $\phi$ is the ratio of $[S_{b0}]$ to $[S_r]$, and represents the kinetic accessibility of cellulose.

$\phi$ was estimated by the following expression:

$$\phi = \beta \frac{[E]_0^{\beta}}{[E]_r}$$

(27)

$[E]_0$ denotes initial substrate concentration. At low $[E]_0$, $v_0$ is directly proportional to $[E]_0$ (i.e. $v_0 = k[E]_0$) and at high concentrations $v_0$ is constant. The intersection of $v_0 = k[E]_0$ and $v_0 = \text{constant}$ gives $[E]_0$. $\beta = 39$ is the number of cellobiosyl units covered by an adsorbed CBH.

The results showed that $\phi$ can be different for different cellulases for the same substrate, e.g. for Avicel, $\phi$ was 0.014 for Cel7B but only 0.0012 for Cel7B. The order of magnitude of $\phi$ and $F_a$ is the same: $F_a = 0.002$ and $\phi = 0.0012-0.014$ for four different enzymes.

The importance of productive adsorption can be illustrated by a simple analysis of the data from Zhang and Lynd (2005), and Hong et al. (2007):

Accessible fraction of the $\beta$-glycosidic bonds in Whatman Filter paper (as calculated by Eq. (25)) ~0.0095, DP ~ 2000. Therefore:

$$[C_x] = \frac{1}{2000} [C_{b0}] = 0.0005^* [C_{b0}]$$

(28)

and $[C_{b0}] = 0.0095^* [C_{b0}]$

(29)

where $[C_x]$ is the concentration of reducing ends, $[C_{b0}]$ is the concentration of all $\beta$-glycosidic bonds, $[C_{b0}]$ is the concentration of accessible $\beta$-glycosidic bonds.

If all the chain ends are occupied at maximum adsorption, there would still be a large fraction of non-productively bound cellobiohydrolase given by:

$$\frac{[C_{b0}] - [C_x]}{[C_{b0}]} = 0.0095 - 0.005 - 0.95$$

(30)

As cellulose chains are hydrolyzed, the chains located below, which were not exposed to cellulases, can undergo hydrolysis. Based on this idea, accessibility parameters have been included in the rate equations (Al-Zuhair, 2008; Converse and Optekar, 1993; Gan et al., 2003; Wood, 1975). It is not clear whether it is possible to classify a part of the substrate in just two categories: accessible and inaccessible. Accessibility as a substrate property could possibly be a continuous variable.

3.5. Role of fractal kinetics in cellulase kinetics

Fractal kinetics is said to occur when reactions take place in spatially constrained media; such reaction conditions give rise to non-uniformly mixed reaction species, apparent rate orders, and time-dependent rate constants (Anacker and Kopelman, 1987; Kopelman, 1986; Kopelman, 1988). Since cellulase hydrolysis of insoluble cellulosic substrates can be thought of as a one-dimensional heterogeneous reaction along a cellulose fiber, it can result in fractal kinetics. Though reactions occurring on a supported catalyst can be modeled using Langmuir–Hinshelwood kinetics (Fogler, 2005), fractal kinetics must be considered for catalytic reactions involving diffusion of two species (for bimolecular reactions) on the non-ideal substrate surfaces (surfaces with obstacles resulting in segregation of species, non-uniform concentrations).

Example of a simple bimolecular reaction, occurring on a catalyst surface modeled by Langmuir Hinshelwood kinetics, is shown below.

$$A + S \xrightarrow{k_1} AS$$

(31)

$$B + S \xrightarrow{k_2} BS$$

(32)

$$AS + BS \xrightarrow{k_3} CS + S$$

(33)

$$CS \xrightarrow{k_4} C + S$$

(34)

where A and B are reacting species, C is product, S is a vacant adsorption site on the substrate.

If the surface reaction step is rate limiting, and the substrate surface is ideal, permitting free diffusion of the species, uniform mixing and no obstacles, we get the following expression for the rate:

$$r = \frac{S_i (k_i K_A C_A C_B - k_{-1} K C_C)}{(1 + K_A C_A + K_B C_B + K_C C_C)^2}$$

(35)

where $r$ denotes the rate, $S_i$ is the total site concentration, $C$ denotes concentration of the species in the subscript, $K_A = k_i/k_{-1}$, $K_B = k_2/k_{-2}$ and $K_C = k_4/k_{-4}$.

Michaelis–Menten kinetics in fractal media was first studied using the power law formalism (Savageau, 1995), where the classical enzyme catalysis reaction (Eq. (36)) in fractal media was described by apparent rate orders (Eqs. (39) and (40)).

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P$$

(36)

where $E$ is enzyme, $S$ is substrate, ES is enzyme–substrate complex, $P$ is product, $k_1$ is the forward rate constant for the association of the enzyme and substrate, $k_{-1}$ is the dissociation constant of the enzyme–substrate complex and $k_2$ is the product formation rate constant.

Classical equations —

$$\frac{d(ES)}{dt} = k_1 E S - (k_{-1} + k_2) (ES)$$

(37)

$$\frac{dP}{dt} = v_p = k_2 (ES)$$

(38)
Power law equations –

$$\frac{d(ES)}{dt} = \alpha_1 \cdot E^{1-g_1} \cdot S^{g_2} - (\beta_1 + \alpha_2)(ES)$$  \hspace{1cm} (39)

$$\frac{dP}{dt} = v_p = \alpha_2(ES)$$  \hspace{1cm} (40)

where $v_p$ is the product formation rate, $\alpha_1$, $\alpha_2$, and $\beta_1$ are new constants introduced for the power law formulation, $g_1$, and $g_2$ are the apparent rate orders with respect to $E$ and $S$.

Using Monte Carlo simulations, the classical enzyme kinetics scheme (Eq. (36)), has been studied in two dimensions in the presence of surface obstacles by Berry (2002). The fractal nature of the reaction system was shown to increase as the obstacle density was increased. $k_1$ (rate constant of a bimolecular reaction requiring the diffusion of enzyme and substrate on the surface) was shown to decrease with time, whereas $k_{-1}$ and $k_2$ were time-invariant, as the unimolecular reaction did not require diffusion. It was also shown that the quasi-steady state assumption cannot be applied in these conditions.

Enzymatic hydrolysis of lignocellulosic biomass is a heterogeneous reaction since it occurs on the substrate surface (large enough to accommodate a large number of enzyme molecules). After adsorption, cellulases have to diffuse on the surface of the substrate to reach the reactive sites (a chain end in the case of cellobiohydrolases). The inaccessible and non-reactive portions of the substrate can be considered as obstacles increasing the fractal character of the hydrolysis reaction. The first work to study cellulose hydrolysis by fractal kinetics was performed by Väljamäe et al. (2003). Using an empirical first-order product formation equation for cellobiose production (Eq. (41)), the parameter $h$, which represents the fractal dimension, was shown to increase with increasing substrate concentration for Cel7A core protein (catalytic domain only) plus Cel5A endoglucanase (0.1 to ~0.45) but to decrease for Cel7A intact protein plus Cel5A endoglucanase (0.6 to ~0.35).

$$P(t) = |S|_0 \left(1 - \exp(-k^*t^{1-h})\right)$$  \hspace{1cm} (41)

where $P(t)$ is the product concentration at time $t$, $|S|_0$ is the initial substrate concentration, $k^*$ is the reaction order constant, and $h$ is time.

It was thus concluded that the intact Cel7A acts in a 2-D surface phenomenon, where diffusion time would be expected to increase with increasing substrate concentration. Similarly, the action of the Cel7A core (catalytic domain) was stated to be a 3-D phenomenon since the diffusion time decreases with increasing substrate concentration.

Contrary to the classical enzyme reaction scheme, the product formation step can also be diffusion-controlled since cellobiohydrolases have to process along the cellulose chain while cleaving β-1,4-glycosidic bonds. This was incorporated in the study by Xu and Ding (2007) who derived the following equation:

$$k_2[E]^{1-f} / (1-f) = [P] - K_m \ln \left(1 - \frac{[P]}{[S]}\right)$$  \hspace{1cm} (42)

where $f$ is the fractal dimension, $k_2$ is the product formation rate constant, $[E]$ is the enzyme concentration, $[P]$ is the product concentration, $[S]$ is the substrate concentration, and $K_m$ is the Michaelis constant. The spectral dimension $d_s$ of a bimolecular reaction is defined by $d_s = 2(1-f)$ (Kopelman, 1988). Values of $f$ were found to be 0.44 ($d_s = 1.12$) and 0.22 ($d_s = 1.56$) for T. reesei Cel7A and H. insolens Cel7A respectively, implying a higher processive action for the T. reesei Cel7A. The effect of overcrowding of the enzymes (referred to as ‘jamming’) was also studied by the use of the following equation:

$$\left(1 - \frac{E}{J[S]}\right) k_2[E]^{1-f} / (1-f) = [P] - K_m \ln \left(1 - \frac{[P]}{[S]}\right)$$  \hspace{1cm} (43)

where $j$ is the jamming parameter. The jamming parameter was found to be around 0.0004.

The above-mentioned two works are only semi-quantitative. They have, however, helped in understanding the role of fractal kinetics in enzymatic cellulose hydrolysis.

There is no conclusive evidence on whether enzyme diffusion on the cellulose surface is rate-limiting for the cellulose hydrolysis process or not. By measuring the diffusion rates of Cellulomonas fimi cellulases on Valonia ventricosa microcrystalline cellulose, Jervis et al. (1997) concluded that the surface diffusion of enzymes was unlikely to be rate-limiting. According to the diffusion rates measured, each cellulase traverses several hundred lattice sites in a minute. These were compared with the hydrolysis rates of C. fimi endoglucanase (CenA) on bacterial microcrystalline cellulose (BMCC) – 0.23 mol glucose/mol enzyme/min (Meinke et al., 1993), which were lower than the diffusion rates. However, as the authors have stated, the importance of the diffusion step also depends on how the hydrolysable sites on the substrate are distributed. The substrate used in this work was highly crystalline; for other cellulosic substrates such as Avicel or Solka Floc, and those consisting of lignin and hemicellulose, it is possible that substrate heterogeneity and partial crystallinity result in rate-limiting diffusion rates. Since jamming occurs when there is overcrowding of cellulases on the cellulose surface, it would be valuable to observe how the hydrolysis rates vary as the amount of adsorbed cellulase increases. Igarashi et al. (2006) measured the hydrolysis rates and specific activity of Cel7A from T. viride as its surface density was increased on cellulose samples from Cladophora and Halocynthia. The hydrolysis rates went through a maximum, whereas the specific activity declined continuously; this was attributed to overcrowding of enzymes on the substrate surface.

As of now, it cannot be concluded which of the above mentioned rate limitations are predominant. While the role of enzyme deactivation, biphasic composition of the substrate, substrate reactivity, and substrate accessibility have long been stated to play a major role, fractal kinetics and jamming have only recently been shown to be important (Bommarius et al., 2008; Väljamäe et al., 2003; Xu and Ding, 2007). In addition to the above-mentioned causes for the declining rates (Section 3.1 to 3.5), decrease in synergism (Ooshima et al., 1991) and inhibition due to lignin (Mansfield et al., 1999; Zhang and Lynd, 2004) have also been reported to reduce cellulase activities on cellulose. According to Ooshima et al. (1991), the decrease in specific activities of the adsorbed enzymes (with conversion) can be explained by the decrease in synergism between endoglucanase and exoglucanase resulting from a change in the ratio of their adsorbed quantities. Modeling synergism and lignin contribution are discussed in the subsequent sections.

4. Modeling synergism of cellulase components

A mixture of cellulase components, cellobiohydrolases and endoglucanases, has higher activity than the individual components alone (Beldman et al., 1988; Fujii and Shimizu, 1986; Gusakov et al., 2007; Henriassat et al., 1985; Klemen-Leyer et al., 1996; Nidetzky et al., 1994b; Schell et al., 1999; Wood and McCrae, 1978; Woodward et al., 1988a; Woodward et al., 1988b). Modeling synergistic kinetics of the cellulases requires separate mathematical expressions for the individual components and the inclusion of cellulose chain ends as a variable in the model. The earliest of such models was proposed by Suga et al. (1975) for exo and endo-enzyme depolymerization of
polysaccharides based on the Michaelis–Menten scheme. This model was extended by Okazaki and Moo-Young (1978) to include product inhibition and β-glucosidase activity. Based on these theoretical studies, Dean and Rollings (1992) developed a model that was inconsistent with the experimental data at longer times. The following data were analyzed: conversion, polydispersity of polysaccharides, synergism, weight-averaged and number-averaged molecular weights of polysaccharides. Substrate and product inhibition, and enzyme deactivation were stated to be possible causes for the lesser predictive capability of the model at longer times. It is also possible that the model class by itself is not correct, therefore, as the authors themselves state, the above mentioned additional kinetic factors need to be incorporated in the models to ascertain the validity/invalidity.

Using substrate concentration as the only substrate variable, Fujii et al. (1981) developed a model where the endo and exo activities were represented by Michaelis–Menten expressions. The model was evaluated for carboxymethyl cellulose and hydroxyethyl cellulose (Fujii and Shimizu, 1986). Another Michaelis–Menten based model for synergism was proposed by Niedetzky et al. (1994b) where an additional term for synergism was added to the equation:

$$v(E_1, E_2) = v(E_1) + v(E_2) + v_{\text{syn}}(E_1, E_2)$$  \((44)\)

where \(v(E_1, E_2)\) is the hydrolysis rate in the presence of two enzymes \(E_1\) and \(E_2\), \(v(E_1)\) and \(v(E_2)\) are the individual hydrolysis rates, and \(v_{\text{syn}}(E_1, E_2)\) is the synergistic hydrolysis rate. However, these models based on the Michaelis–Menten scheme have limitations, as discussed in the Section 2.2 ‘Michaelis–Menten based models’.

Converse and Optekar (1993) took into account enzyme adsorption, degree of polymerization, and accessibility of the substrate to model cellulose hydrolysis by cellobiohydrolase and endogluccanase. The model matched the experimental data well till a conversion level of approximately 40% (data from Woodward et al. (1988b)). The adsorption and DP variations were not, however, validated by experiments. The degree of synergism, which was shown to go through a maximum as the cellulase concentration increased, has been explained by the ‘substrate inhibition’ phenomenon (Väljamäe et al., 2001). At low surface coverage of the substrate (a condition achieved at high substrate concentration relative to enzyme), synergism is low as cellobiohydrolases do not benefit from the new chain ends created by endoglucanases. Substrate inhibition was also observed by Liaw and Penner (1990), and Huang and Penner (1991), but no implications of synergism were discussed. At high surface coverage (low substrate/high enzyme concentrations) competition among enzyme species for adsorption results in a decrease in synergism. Fenske et al. (1999) used Monte Carlo simulations for an enzyme that featured both endo and exo activity. Hydrolysis rates were shown to be lower at low surface coverage of the substrate due to the partial endo activity of the enzyme and went through a maximum as the substrate concentration increased. This phenomenon was termed ‘auto-synergism’.

A deeper understanding of enzyme synergism is needed to optimize the mixtures of endoglucanases and cellobiohydrolases. Since the adsorbed amount of cellulases is susceptible to change along conversion, it is crucial to study these variations and their implications on synergism. Experimental data that corroborate model predictions on variations in DP and chain size distributions are required to get accurate parameter values associated with these substrate properties. So far no work has successfully achieved such a validation. Dean and Rollings (1992) attempted to validate their model for non-cellulosic substrates (dextran-polysaccharide with α-1,6-glycosidic linkages) but were unable to match the experimental data at longer residence times. As the reaction proceeded, a change in the type of pattern in the size distribution was observed (Klemán-Leyer et al., 1994; Klemán-Leyer et al., 1996; Mansfield and Meder, 2003; Pala et al., 2007; Rammos et al., 1993). This shows that the susceptibility of a substrate to enzymatic attack can vary with chain size. The complexity associated with the accessibility of the available chain ends on the heterogeneous substrate is clearly a key issue that needs to be addressed before depolymerization models become informative.

5. Models of pure cellulosic substrates and lignocellulosic substrates

Lignin reduces the accessibility of cellulose to cellulases and also adsorbs cellulases, resulting in lower hydrolysis rates (Mansfield et al., 1999). The effect of lignin content is also evident from numerous empirical models (see Table 1A). Since the presence of lignin can significantly affect the hydrolysis rates, models developed for pure cellulosic substrates cannot be extended to substrates having high lignin content. For example, in the presence of lignin, a two-phase model might be applicable, whereas for pure cellulosic substrate it is not apparent. Adsorption of cellulase and β-glucosidase onto lignin has been incorporated into a few models with rate equations (Shao et al., 2005a) (see Eqs. (13) and (14)) and Langmuir isotherms (Ljunggren, 2005; Pettersson et al., 2002; Philippidis et al., 1993; Philippidis et al., 1992; Zheng et al., 2009). It was shown by Zheng et al. (2009) that their model did not match the experimental data if the negative role of lignin was ignored. Shin et al. (2006) accounted for the presence of non-cellulosic materials in steam-exploded wood by including an inhibition parameter. It has been shown that cellulases having similar activity on pure cellulosic substrates can have different affinities for lignin (Berlin et al., 2005). Synergism results for pure cellulosic substrates might also be different for more realistic substrates since the affinity of various cellulosic materials for non-cellulosic parts can vary. Changes in crystallinity can also be affected by lignin (Zhang and Lynd, 2004), and hence the observation of crystallinity variations along conversion must be interpreted carefully. The extent to which crystallinity limits the enzymatic conversion of biomass into sugars can depend on the lignin level and vice-versa (Zhu et al., 2008). Since lignin is not degraded by cellulases, it can act as a barrier resulting in stoppage of the enzymes on the substrate. In terms of fractal kinetics, lignin and hemicellulose act as obstacles and hence increase the fractal nature of the reaction system.

Deeper understanding of the role of lignin in enzymatic digestion of lignocellulosic and its interaction with enzymes is needed not just for improving pretreatment technologies but also for engineering enzymes that have lesser affinity for lignin (Berlin et al., 2005). This is possible through quantification and modeling of lignin contribution in various steps of the hydrolysis process.

6. Conclusions and outlook

Cellulase hydrolysis of cellulose is a reaction in heterogeneous medium. Classical homogenous enzyme catalysis is modeled by Michaelis–Menten kinetics and heterogeneous catalysis on a catalyst support, by Langmuir–Hinshelwood kinetics. Cellulase kinetics on insoluble lignocellulosic substrates is a combination of the above two kinds of reactions and also involves other factors (product inhibition, enzyme deactivation, substrate crystallinity, substrate accessibility changes, substrate reactivity changes, fractal nature of the reaction, changes in enzyme synergism, lignin inhibition), which result in retarding the rates at higher degrees of conversion. While the models in literature have not pinpointed the exact mechanism of enzymatic action on lignocellulosic materials, they have helped in understanding the various factors that are at play. Additional insight will be made possible by models consisting of the major substrate and enzyme properties (substrate-concentration, DP, accessible fraction, size-distribution of chains, crystallinity; enzyme-concentration, synergistic/competitive factors, and adsorbed concentration of
individual components). However, due to the increase in the number of parameters, such models need to be validated with experimental data other than conversion-time profiles to distinguish between the various causes of decreasing rates. It is clear from the research reviewed in this article that adsorption, substrate reactivity, and accessibility can change along conversion. Therefore, their dynamic nature must be taken into consideration when building models. The range of conversion for checking the predictive ability of a model is also important, since major slowdowns are observed at high conversions. Only one-third of the models reported have been validated with data beyond 70% conversion (see Tables 1A–D).

Improvements in enzyme catalysis have mainly been guided by the engineering of the active site or amino acid residues identified as playing an important role. In the case of cellulases and their kinetics on insoluble lignocellulosic substrates, rate limitations cannot be explained solely by active-site considerations, mostly because of the heterogeneity of the substrate. Information regarding the catalytic domain, the binding domain, and the linker region (the three domains of a cellulase) through advances in structural biology will certainly contribute to a more complete understanding of the operation of cellulases at the molecular level. Additionally, to significantly improve the enzymatic process, contributions of the various substrate characteristics need to be quantified to specifically target the enzyme and substrate features that need improvement.

Acknowledgment

The authors thank the Chevron Corporation for the financial support.

References


Beldman G, Voragen AGJ, Rombouts FM, MFS-v Leeuwen, Pilnik W. Adsorption and substrate features that need improvement.

Beldman G, Voragen AGJ, Rombouts FM, Pilnik W. Synergism in cellulose hydrolysis by cellulases at the molecular level. Additionally, to signify...


