Rheology of Lignocellulose Suspensions and Impact of Hydrolysis: A Review

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Abstract White biotechnologies have several challenges to overcome in order to become a viable industrial process. Achieving highly concentrated lignocellulose materials and releasing fermentable substrates, with controlled kinetics in order to regulate micro-organism activity, present major technical and scientific bottlenecks. The degradation of the main polymeric fractions of lignocellulose into simpler molecules is a prerequisite for an integrated utilisation of this resource in a biorefinery concept. The characterisation methods and the observations developed for rheology, morphology, etc., that are reviewed here are strongly dependent on the fibrous nature of lignocellulose, are thus similar or constitute a good approach to filamentous culture broths. This review focuses on scientific works related to the study of the rheological behaviour of lignocellulose suspensions and their evolution during biocatalysis. In order to produce the targeted molecules (synthon), the lignocellulose substrates are converted by enzymatic degradation and are then metabolised by micro-organisms. The dynamics of the mechanisms is limited by coupled phenomena between flow, heat and mass transfers in regard to diffusion (within solid and liquid phases), convection (mixing, transfer coefficients, homogeneity) and specific inhibitors (concentration gradients). As lignocellulose suspensions consist of long entangled fibres for the matrix of industrial interest, they exhibit diverse and complex properties linked to this fibrous character (rheological, morphological, thermal, mechanical and biochemical parameters). Among the main variables to be studied, the rheological behaviour of such suspensions appears to be determinant for process efficiency. It is this behaviour that will determine the

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equipment to be used and the strategies applied (substrate and biocatalysis feed, mixing, etc.). This review provides an overview of (i) the rheological behaviour of fibrous materials in suspension, (ii) the methods and experimental conditions for their measurements, (iii) the main models used and (iv) their evolution during biocatalytic reactions with a focus on enzymatic hydrolysis.

Keywords Fibre suspension · Hydrolysis · Lignocellulose · Paper pulp · Rheology · Viscosity · Yield stress

Nomenclatures

Abbreviation
BAG   Sugarcane bagasse
CoSt   Corn stover
MCC   Microcrystalline cellulose
MFC   Microfibril cellulose
NCC   Nanocrystalline cellulose
PP   Paper pulp
RVA   Rapid viscosity analyser
WhSt   Wheat straw
WP   Whatman paper

Greek Letters
$\dot{\gamma}$ Shear rate (s^{-1})
$\dot{\gamma}^*$ Complex shear rate (s^{-1})
ϕ Volume fraction (/)
ϕ_c Critical volume fraction (/)
ϕ_m Maximum volume fraction (/)
ϕ_{eff} Effective volume fraction (/)
$[\mu]$ Intrinsic viscosity (/)
ρ Density (kg m^{-3})
ρ_s Substrate density (kg m^{-3})
μ Viscosity (Pa s)
μ_0 Initial viscosity (Pa s)
μ_∞ Final viscosity (Pa s)
μ_s Suspending viscosity (Pa s)
μ_{rel} Relative viscosity (/)
τ Shear stress (Pa)
τ_0 Yield stress (Pa)
ξ Ionisation energy (mV)

Latin Letters
C*   Critical substrate concentration (gdm L^{-1})
Cm   Mass concentration (gdm L^{-1})

CrI   Crystallinity (%)
d Particle diameter (m)
$d_{4,3}$ Volume mean diameter (m)
n Power-law index (/)
K_p Power constant (/)
K_s Metzner-Otto coefficient (/)
k Consistency index (Pa s^n)
k_c Cosson constant (Pa$^{1/2}$ s$^{1/2}$)
N_p Power number (/)
N_{po} Power number in turbulent flow (/)
Re   Reynolds number (/)
Re_g Generalised Reynolds number (/)
Re*   Rieger and Novak Reynolds (/)
G′   Elastic modulus (Pa)
G″   Viscous modulus (Pa)
G*   Complex modulus (Pa)
N Mixing rate (round per second)
P Power consumption (W)

Contents

1 Introduction

For a number of years now, environmental preoccupations and energy control are at the heart of scientific and social debate. Behind the increase of energy consumption, and the depletion of fossil fuel resources, the developments of biorefineries and industrial biotechnology in general are of the greatest importance to make concrete the vision of an economy based on bioresources. The important role of white bio-technology is to replace petro-derived chemicals by those produced from biomass.

The main biomass resources include short rotation forestry (willow, poplar, *euca-lyptus*), wood-based industries (pulp and paper industry, forest residues, sawmill and construction/industrial residues, etc.), sugar crops (sugar beet, sweet sorghum), starch crops (maize, wheat, Jerusalem artichoke, etc.), herbaceous lignocellulosic crops (*miscanthus*), oil crops (rapeseed, sunflower), agricultural waste (straw, slurry), municipal solid waste and refuse, and industrial waste (residues from food industry). These fractions can be used directly as desired biochemical or can be converted by thermal, mechanical, chemical, enzymatic and/or microbial approaches. Among these large users of lignocellulosic resources, the pulp and paper industry holds a strategic position. Pulp and paper industry [1] is able to provide a tried and tested industrial model for the processing of lignocellulosic biomass into pre-treated cellulose pulps. The pulp product of this industry is appropriate for modern biorefining, because it displays low lignin content, is free of inhibitory compounds that can perturb fermentations and is devoid of microbial contaminants. Cellulose biomass thus provides an abundant low-cost resource that has the potential to support large-scale production of fuels and chemicals via biotechnological routes [2].

The first hydrolysis methods used were chemical, but they are less competitive at the moment, because of the cost of reagents and the formation of numerous by-products and inhibitory compounds. They are now challenged by enzymatic methods, which allow more specific hydrolysis at higher yields and in less severe
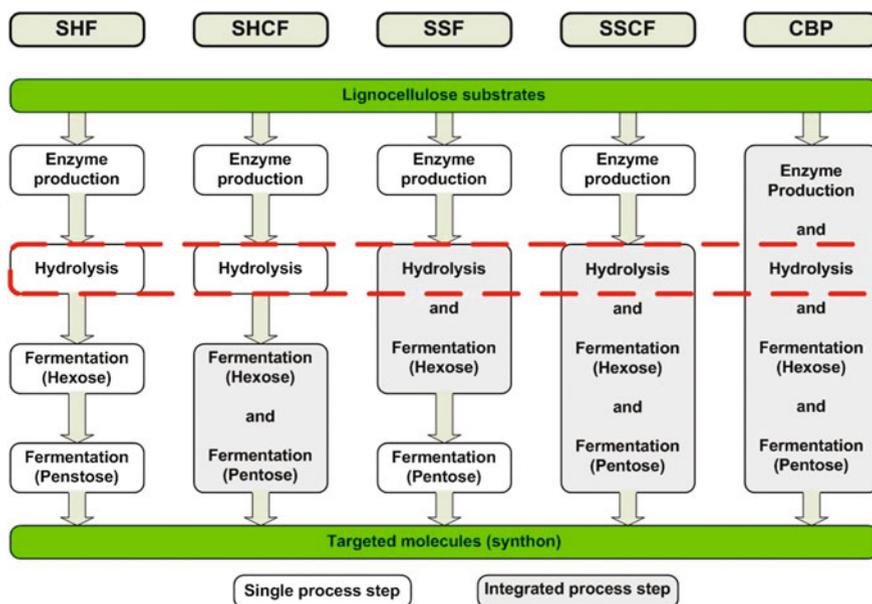
Fig. 1 Various configurations of biologically mediated processing steps during the biocatalytic conversion of lignocellulose and highlight on hydrolysis step with and without interaction with cell cultures. Abbreviations: separate hydrolysis and fermentation (*SHF*), separate hydrolysis and co-fermentation (*SHCF*), simultaneous saccharification and fermentation (*SSF*), simultaneous saccharification and co-fermentation (*SSCF*), and consolidated bioprocessing (*CBP*)

conditions [3]. An enzyme-based process can be divided into four principal steps (Fig. 1): (1) pre-treatment: due to the recalcitrant nature of native lignocelluloses, physical/chemical methods must be applied to generate an enzymatically convertible material; (2) enzymatic hydrolysis: where the cellulose and hemicelluloses are enzymatically degraded into sugar monomers; (3) fermentation: sugar monomers are converted into target molecules, often by yeast; and (4) distillation: to recover products [4–6]. In order to produce sugars, biomass is pre-treated with physical and/or thermo-chemical and possibly biological methods to reduce the size of the feedstock and to open up the plant structure. The cellulose and the hemicellulose portions are broken down (hydrolysed) by enzymes to yield simple sugars that are then fermented into target products by specific strains of micro-organisms. Currently, this biochemical route is the most commonly used [7].

To convert lignocellulose biomass into molecules of interest, the key steps focus on the pre-treatment technique and the conversion into fermentable sugars. The major challenges for cellulose-based products concern cost reduction for production, harvest, transportation and upfront processing in order to make cellulose-based products competitive with grain-based products [8]. Therefore, a better scientific understanding and ultimately technical control of these critical biocatalytic reactions, which involve complex matrices and high solids content, are currently the major challenges that must be won if biorefining operations are to become commonplace. Among the main parameters studied, the rheological behaviour of the hydrolysis suspension and the morphology and size of fibres stand out as major determinants of process efficiency and determine the equipment to be used and the strategies to be applied [9]. Indeed, suspensions exhibit a very wide range of rheological behaviour, and numerous examples can be found to illustrate shear-thinning/shear-thickening behaviour, viscoplasticity with observation of yield stress, elasticity or thixotropy. A lot of parameters influence the nature and the intensity of these non-Newtonian characteristics: the concentration, the particle size and the morphology of the objects, the nature and the magnitude of the particle interactions, etc. Many industries, especially when bioprocesses are involved, are confronted with these behaviours which can sometimes drastically affect hydrodynamics and transfer efficiency (mass or/and heat). It is then of crucial interest to explore the rheology of the suspension to ensure optimal process implementation and the right choice of equipment [10]. It requires a thorough knowledge of the rheological behaviour of the substrate suspensions.

These suspensions, after or simultaneously with hydrolysis, are converted into target molecules by various micro-organisms whether prokaryotes or eukaryotes. The physico-bio-chemical complexities of the broth resulting from these multiphase and dynamic media lead to additional complexity compounded by the fibrous morphology. The rheological behaviour is sensitive to multifactorial parameters (medium compositions, molecules produced, morphology and physiology of the micro-organisms present). Considering flow properties of suspensions, strong analogies between the growth of filamentous fungus cultures [11] and hydrolysis of complex lignocellulose matrices can be made concerning the rheological behaviour, hydrodynamics and transfers (definition of significant morphological criteria,

impact of concentration and morphology, main rheological parameters: shear-thinning behaviour, yield stress). In this paper, the methods and the models related to the rheological behaviour of complex lignocellulosic suspensions are similar to or adapted from filamentous micro-organism suspensions. For simultaneous or consolidated bioprocesses, a good knowledge of the physico-bio-chemical properties of suspensions (lignocellulose materials, micro-organisms) is required to define accurate operating conditions (transfer efficiency) for substrate release kinetics (biocatalytic hydrolysis) and micro-organism metabolism (growth, production) kinetics.

A database interrogation was conducted to highlight scientific production focusing on "lignocellulosic materials, flow properties and biocatalytic degradation" for the last 40 years. In this aim, four specific profiles were defined:

- Profile 1: (Rheo* OR visco* OR newt*) AND (suspension* OR dissolution*) AND cellulose*
- Profile 2: (Rheol* OR visco*) AND fiber* AND cellulose*
- Profile 3: (Rheol* OR visco*) AND (paper pulp* OR pulp suspension*)
- Profile 4: Bioproce*

(* stands for truncation option, i.e.: visco* stands for viscosity, viscosimetry, viscous…).

The first and second profiles focus on the rheological behaviour of cellulose/fibre suspensions. The third one scrutinises specifically the pulp and paper industry, while the last one describes the global trends in biotechnological process. Figure 2 presents the chronological progression for these profiles with the number of publications per year. We observed a regular and remarkable increase since 1990 for all these profiles. This tendency is a medium-term consequence of the successive petroleum crises in the seventies. It is noticeable that the interest for the rheological behaviour of potential substrates/suspensions for second-generation biofuels has increased in the same way as research and developments in bioprocessing.

To carry out an overview of lignocellulose suspension rheology and its evolution during hydrolysis, the different published results reporting the methods of investigation and the results obtained (rheological behaviour and its relationship with different physical parameters: particle size, mechanical properties of fibre) are presented.

2 Lignocellulose: Nature and Characterisation

Considering rheological behaviour, the scientific publications simultaneously investigating physical properties and biochemical composition during suspension and/or hydrolysis have been extracted and are reported in Table 1. It is noticeable that this extraction leads to a restricted number of publications and limited information. Physical and biochemical properties are defined by particle diameter, aspect ratio, density, crystallinity, surface properties and biochemical compositions but are rarely integrally reported. Various substrates were studied and could be classified
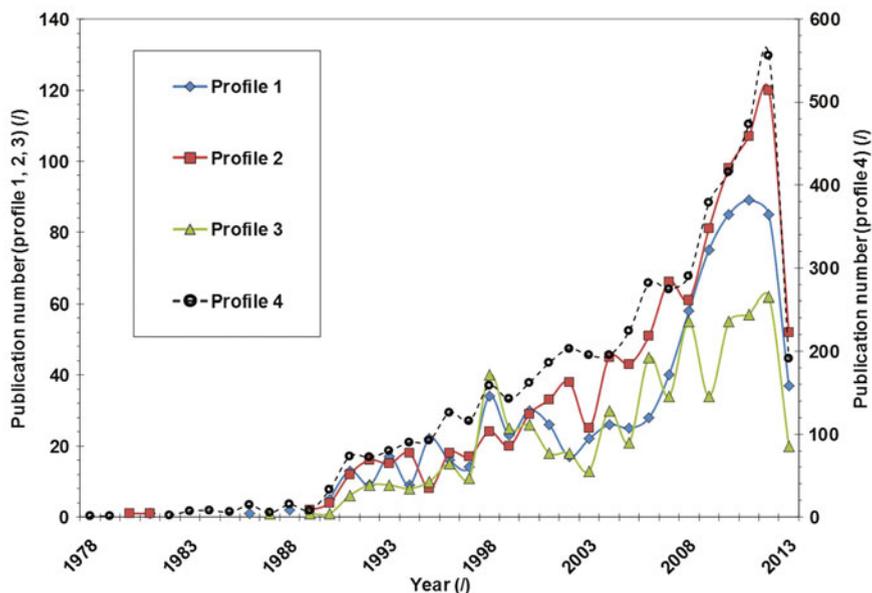
Fig. 2 Number of publications per year since 1978 (Profile 1: (Rheo* OR visco* OR newt*) AND (suspension* OR dissolution*) AND cellulose*; Profile 2: (Rheol* OR visco*) AND fiber* AND cellulose*; Profile 3: (Rheol* OR visco*) AND (paper pulp* OR pulp suspension*); Profile 4: Bioproce*)

into two groups: cellulose (simple matrices) and lignocellulose (complex matrices). Cellulose matrices are mainly nanocrystalline, microcrystalline and microfibril cellulose (NCC, MCC and MFC) with diameters ranging from 0.1 μm for NCC up to 100 μm for MCC. For complex matrices, a great variety of lignocellulosic substrates are derived from woody materials or by-products of agriculture (softwood pulp, hardwood pulp, wood powder, corn stover, bagasse sugarcane, paper). Such complex matrices have been processed with specific pre-treatments which confer various and singular characteristics with a strong influence of the fibrous morphology. These critical physical properties were unequally reported although they make a significant contribution to the rheological behaviour of the suspension.

2.1 Physical Characterisation of Fibres

Among the main parameters to be studied, the rheological behaviour of the hydrolysis suspension and the fibre size and morphology stand out as major determinants of the process efficiency. They are the key elements for the choice of the equipment and the feeding/hydrolysis strategy. However, numerous other parameters related to fibre affect bioprocess performances:

Table 1 Scientific publications linked to rheology, physico-chemical characterisations of lignocellulosic matrices and enzymatic hydrolysis

	Matrices	Publication	Study operations		Physical properties					Biochemical compositions				
			Suspension	Hydrolysis	d_m (μm)	A. ratio	ρ (kg/m^3)	ζ (mV)	CrI	% d_m	Cellu.	Hemi.	Lig.	Ash
Cellulose	NCC	Araki et al. [12]	x	–	0.18 ± 0.075	51 ± 21	–	–	–	–	–	–	–	–
	NCC	Boluk et al. [13]	x	–	0.18 ± 0.06	30 ± 14	–	-62.8	–	–	–	–	–	–
	NCC	Gonzalez-Labrada and Gray [14]	x	–	0.117	29	–	-47	–	–	–	–	–	–
	NCC	Lu et al. [15]	x	–	0.87	5.4	1560	-58	–		–	–	–	–
	MCC	Luukkonen et al. [16]	x	–	60	–	–	–	–	–	98	0	0	2
	MCC	Tatsumi et al. [17]	x	–	0.72–350	21–450	–	–	–	–	–	–	–	–
	MCC	Bayod et al. [18]	x	–	30–35	1.5–7	–	–	–	–	–	–	–	–
	MCC	Horvath and Lindstrom [19]	x	–	20–30	–	–	–	–	–	–	–	–	–
	MCC	Um and Hanley [20]	x	x	91	–	–	–	–	–	88	–	–	–
	MCC	Tatsumi et al. [21]	x	–	1.7	22	–	–	–	–	–	–	–	–
	MCC	Tozzi et al. [22]	x	–	24–27	–	–	–	–	90–92	68–72	7–7.8	4–5	0.15
	MFC	Lowys et al. [23]	x	–	–	–	–	-20	–	–	85	11	0	4
	MFC	Agoda-Tandjawa et al. [24]	x	–	10	80–500	–	–	11–13	–	93	–	–	3.6
	MFC	Saarikoski et al. [25]	x	–	–	–	–	–	–	–	–	–	–	–

(continued)

Table 1 (continued)

	Matrices	Publication	Study operations		Physical properties					Biochemical compositions				
			Suspension	Hydrolysis	d_m (µm)	A. ratio	ρ (kg/m³)	ζ (mV)	Crl	% d_m	Cellu.	Hemi.	Lig.	Ash
Lignocellulose materials	BAG	Geddes et al. [26]	x	x	$(25–50) \times 10^3$	–	–	–	–	–	–	38	28	–	–
	BAG	Pereira et al. [27]	–	x	<2000	–	1420	–	–	–	43.6	8.75	33.75	–
	CoSt	Pimenova and Hanley [28]	x	–	120	–	–	–	–	–	–	–	–	–
	CoSt	Stickel et al. [29]	x	–	100	1–20	–	–	–	–	–	–	–	–
	CoSt	Viamajala et al. [30]	x	–	–	–	–	–	–	93.5	33.2	21.6	–	–
	CoSt	Dunaway et al. [31]	x	x	–	–	–	–	–	–	60	5	32	3
	CoSt	Dibble et al. [32]	x	x	80–680	–	–	–	–	–	42–60	4.0–24	18–26	–
	WP	Samaniuk et al. [33]	x	x	600	–	–	–	–	100	99	–	–	–
	CoSt		x	x	3000	–	–	–	–	100	50	–	–	–
	PP	Damani et al. [34]	x	–	1180/1290	–	356/538	–	–	–	–	–	–	–
	PP	Chen et al. [35]	x	–	30/15	107/73	–	–	–	–	–	–	–	–
	PP	Blanco et al. [36]	x	–	1000	–	–	–	–	–	–	–	–	–
	PP	Derakhshandeh et al. [37]	x	–	670–2960	–	–	–	–	–	–	–	–	–
	PP	Le Moigne et al. [38]	–	x		–	–	–	46–49	–	90–94.7	2–3.5	1.1–1.8	–
	PP	Chaussy et al. [39]	x	–	1280	–	–	–	–	–	–	–	–	–
	PP	Wiman et al. [9]	x	x	188	–	–	–	–	13	48	3.2	45	–
	WhSt	Szijjarto et al. [40]	–	x	–	–	–	–	–	34	58.9	–	–	–
	Wood	Dasari and Berson [41]	x	x	33–850	–	–	–	–	–	39.7	19.8	–	–
	Wood	Palmqvist and Liden [42]	–	x	<10,000	–	–	–	–	36.5/12.7	50.2/42.9	–	36.8/46	–

NCC nanocrystalline cellulose; *MCC* microcrystalline cellulose; *MFC* microfibril cellulose; *PP* paper pulp; *BAG* sugarcane bagasse; *CoSt* corn stover; *WhSt* wheat stover

- Dimensions (equivalent diameter, thickness),
- Qualitative granulometry: sawdust and coarse particles (buchettes), short and long fibres, and flours,
- Morphology and specific surface areas (aspect ratio, complex shape factor: curvature, waviness and wrinkling),
- Mechanical properties (elastic modulus, tensile yield strength and ultimate elongation),
- Surface properties with electrostatic and non-electrostatic forces (Zeta potential, hydrophilicity/hydrophobicity, interfacial surface energy),
- Density (humid and intrinsic density) and porosity,
- Degree of lignification (correlated to the Kappa index in the pulp and paper industry) and crystallinity.
- Molecular mass, degree of polymerisation

There are various ways to define the size of a particle as a "diameter." Allen [43] listed thirteen possible ways to define an equivalent diameter for a given particle using the sphere as reference. Each definition of an "equivalent diameter" should give the same result when applied to spheres (i.e. diameter). Therefore, most of the particles are not spherical; the knowledge of more than one dimension is required to describe the shape of a particle and the associated distribution. The most useful diameter is the diameter of the volume equivalent sphere, d_v, which corresponds to the diameter of the sphere having the same volume. For irregular shapes, the magnitude of the equivalent diameter is affected by the measurement methods, which must then be selected with a particular care.

Because of the non-uniform size of particles, variations in a population are described by a size distribution. Frequency distributions or cumulative distributions are conventionally used. Many characterising techniques (microscopy, settling velocity, granulometry, morphometry, diffraction light scattering and focus beam reflectance) give distributions based on the number, length, surface area or volume of the particles. Considering the complexity of particle shapes and according to the properties highlighted, it is important to define a mean diameter (and standard deviation describing the width of the distribution around this average trend) for a given particle population. For instance, average diameters are defined as follows:

$$d_{p,q} = \left[\frac{\sum n_i \cdot d_i^p}{\sum n_i \cdot d_i^q} \right]^{1/p-q} \tag{1}$$

with n_i is the number of particles of diameter d_i, and p and q are the integers ($p = q + 1$ with $q = 0, 1, 2, 3$ for number weighted, length weighted, surface weighted and volume weighted, respectively) [44]. With these notations, $d_{1,0}$ is the number-average diameter, $d_{2,0}$ is the quadratic mean diameter, $d_{3,0}$ is the cube average diameter, $d_{4,3}$ is the mass or volume mean diameter, and $d_{3,2}$ is the area-average diameter or Sauter diameter.

2.2 Rheological Characterisation of Suspensions

Presenting an overview of the scientific literature, this chapter deals with (i) rheometry devices and set-ups for rheological characterisation of lignocellulose suspensions and (ii) rheological behaviour analysis.

2.2.1 Rheological Measurement Devices

Several approaches are suitable for rheological characterisation, and numerous examples of each can be found in the literature concerned with cellulose/lignocellulose suspensions. This is illustrated by studies listed in Table 2. These methods can be classified as a function of device complexity and associated methodologies. The cheapest and easiest-to-use viscometers, i.e. the capillary viscometer and the falling (or rolling) ball viscometer, are suitable for Newtonian fluids but are somewhat difficult to use with unknown non-Newtonian fluids. Their applicability is reduced for fibre suspensions. The works of Luukkonen et al. [16] and of Gonzalez-Labrada and Gray [14] on nano- and microcrystalline cellulose can be mentioned. Fine rheometry analysis was reported by Tozzi et al. [22] using magnetic resonance imaging (MRI) to determine the velocity field in a portion of a cylindrical duct. Completed by the measurement of the pressure drop, the velocity profile brings information on the nature and the characteristics of the fluid. However, this technique is of academic interest but hard to expand to industrial contexts.

The very classical and quite suitable way to obtain rheological characterisation (mostly viscosity, but not only) is the use of rheometers or viscometers equipped with cone and plate, narrow-gap coaxial cylinders or parallel plates. The flow generated is then a simple shearing flow with, except for the parallel plates, a constant shear rate all over the fluid. The shear rate $\dot{\gamma}$ and the shear stress τ are calculated from the rotation frequency and the torque, respectively, and from the characteristics of the geometry. The large-gap coaxial cylinders, which are frequently used in industry, can be considered separately among viscometers as, with a large gap, no assumptions as to the velocity profile can be made (the fluid's rheological characteristics must be known for that). The raw data cannot then be exploited as simply as in the Newtonian case [45, 46]. Coming back to the usual geometries for rheometers and viscometers, they usually require a small volume of fluid, but this advantage turns into a drawback when the size of the objects in suspension is not negligible when compared to a characteristic dimension of the geometry (for instance, a usual gap for parallel plates or concentric cylinders is around 1 mm and is lower for cone and plate geometries). To obtain values of the viscosity from the global measurements performed on a rheometer (torque and angular velocity), the fluid must indeed be considered as a homogeneous medium. It must be borne in mind that this assumption can become erroneous when suspensions are studied.

To overcome this difficulty, the mixing system (MS) of a process, when it exists, can be used as a rheometer, measuring the torque and the rotation rate (or

Table 2 Rheological characterisation of cellulose/lignocellulose suspensions, overview of methodologies used and quantities measured

Authors	Measurement system and mode	Quantities measured
Bennington et al. [45]	Rheometer/concentric cylinders	τ_0 (viscometry)
Damani et al. [34]	Rheometer/parallel plate	G', G"
Araki et al. [12]	Viscometer/double-gap cylinders	μ
Swerin [46]	Rheometer/concentric cylinders	G', G"
Wikström et al. [47]	Rheometer/Rushton-type turbine	τ_y (viscometry)
Tatsumi et al. [48]	Rheometer/cone plate	τ and G', G"
Lowys et al. [23]	Rheometer/cone plate and parallel plates	μ, τ_0 and G', G"
Luukkonen et al. [16]	Capillary viscometer	μ
Tatsumi et al. [17]	Rheometer/parallel plates	μ, τ_0 and G', G"
Chen et al. [35]	Rheometer/parallel plates	μ
Pimenova and Hanley [28]	Viscometer/cone plate and double helical ribbon	μ, τ_0
Bayod et al. [18]	Rheometer/concentric cylinder	μ
Ein-Mozaffari et al. [49]	Viscometer/four-bladed vane	τ_0 (viscometry)
Dasari and Berson [41]	Rheometer/six-bladed vane	μ
Blanco et al. [36]	Mixing system/various mobiles	Torque
Horvath and Lindstrom [19]	Rheometer/parallel plates	τ_0 (oscillations), G', G"
Stickel et al. [29]	Comparison of different methods	τ_0
Knutsen and Liberatore [50]	Rheometer/four-bladed vane (narrow gap)	τ_0 (viscometry), G', G"
Agoda-Tandjawa et al. [24]	Rheometer/parallel plates	μ and G', G"
Derakhshandeh et al. [37]	Viscometer/four-bladed vane + UD velocimetry	μ, τ_0 (viscometry + velocimetry)
Chaussy et al. [39]	Industrial disc refiner	μ
Samaniuk et al. [33]	Mixing system/counter-rotating screw elements	Torque
Wiman et al. [9]	Rheometer/four-bladed vane	μ and G', G"
Gonzalez-Labrada and Gray [14]	Rolling ball viscometer	μ
Saarikoski et al. [25]	Rheometer/concentric cylinder	G', G"
Nguyen et al. [51]	Rheometer/Serrated plates + Mixing system/four-pitched-blade turbine	μ and G', G", Torque
Mohtaschemi et al. [52]	Viscometer/four-bladed vane	μ, τ_0
Tozzi et al. [22]	Poiseuille flow + MRI velocimetry	μ

equivalently the power consumption and the rotation rate), but the difficulty here lies in the complex flow field generated [53]. Determination of viscosity then relies on the previous determination of the power number—Reynolds number characteristic curve of the MS, where the power number Np is a dimensionless number linked to power consumption. This single master curve depends only on impeller/reactor shape and geometry. In the laminar regime (Re < 10–100), the product $N_p \cdot$ Re is a constant, named *Kp,* and a deviation from $N_p \cdot$ Re $= K_p$ indicates the end of the laminar regime. In fully turbulent flow (Re > 10^4–10^5) and for Newtonian fluids, the dimensionless power number N_p is assumed to be independent of mixing Reynolds number and equal to a constant, Np_0. Knowing N_p gives a value of the Reynolds number and an estimation of the viscosity if the fluid is a Newtonian one, and of an equivalent viscosity if the fluid is non-Newtonian. In this last case, interpretation of the rotation frequency and power consumption measurements requires an additional hypothesis that was first proposed by Metzner and Otto [54]. These authors showed that for a large range of shear-thinning fluids represented by a power-law model $(\tau = k\dot{\gamma}^n)$, the equivalent shear rate corresponding to the equivalent viscosity is proportional to the rotation frequency via a K_s coefficient (known as the Metzner-Otto coefficient). In addition, the K_s coefficient depends only on the mixing system characteristics. It is thus sufficient to determine it in a preliminary stage by considering Metzner and Otto's concept [54] or Rieger and Novak's approach [55] based on the generalised Reynolds number, Re* assuming $N_p \cdot$ Re* $= K_p(n)$. In restricted cases, coefficient K_s can be extended to the transition region using a power equation [56]. This online viscosimetry method has been widely used [33, 51, 57, 58]. Note that such a device gives only the viscosity that is to say the shear-thinning/shear-thickening character of the fluid. Nevertheless, such rheometrical measurement systems are attractive as (i) they avoid suspension settling problems (with suitable geometry); (ii) because of the complex flow generated, they elude migration of particles/fibres which are responsible for heterogeneity in the fluid volume; (iii) it is not necessary to take samples (which, if processed, have to be representative and of a negligible volume); and (iv) they can be used online to follow a process.

One can also point out that geometries such as anchor or helical ribbon are proposed for rheometers which are then used as well-characterised mixing systems [40]. They have to be used with the same precautions as for mixing systems. More simply, but also less rich in information, such geometries can also be used in a relative way to follow the time course of global viscosity in some processes [26, 34]. A special geometry proposed for rheometers is the four-bladed (or six-bladed) vane. Never used as a mixing system, its use in rheometers is especially interesting for yield stress fluids to avoid the wall slip effects currently observed for suspensions (formation of a depletion layer caused by migration of fibres away from the wall). This geometry allows precise determination of the fluid behaviour for low shear rate and is thus of special interest for the determination of the yield stress τ_0. Its use for intermediate and high shear rates requires careful analysis when it is assimilated to the geometry of large-gap coaxial cylinders (see [59] for details). Such a geometry was used in a narrow-gap configuration by Knutsen and Liberatore [50] and in a large-gap

configuration by Mohtaschemi et al. [52] for fibrillated cellulose suspensions and by Derakhshandeh et al. [37]. These last authors examined the validity of such geometry in more detail for fibre suspensions by analysing the velocity profile determined by ultrasonic Doppler velocimetry. Stickel et al. [29] proposed a comparison, for a corn stover suspension of a given concentration, of yield stress measurements using the different methods presented here.

The choice of rheometer type then depends on the nature of the fluid tested. Non-classical ways are commonly used to study fluids displaying complex characteristics including large particles, settling problems, slip and time dependency. Geometries that facilitate mixing, such as helical ribbons, anchors and paddles, can overcome settling problems and errors due to slip. Concerning lignocellulose suspensions, the main problems for measurement are heterogeneous suspension, floc formation [35] and particle decantation [51, 60, 61]. Wall slip is currently encountered with suspensions, and the use of surfaces with roughness equal to twofold or threefold the largest particle dimension is recommended [62]. Smooth surfaces, or inadequate roughness, lead to a depletion zone near the wall where the shear rate is higher, thus reducing the shear rate in the bulk fluid and distorting, sometimes significantly, the rheogram as illustrated in Fig. 3. It is thus important to take precautions when this kind of measurement must be carried out and some reports must be considered with caution when nothing is mentioned on this point. Aggregation of fibres is also a crucial point in suspension rheometry. It is caused by the constant and unidirectional shear rate applied to the suspension. An extreme case is presented as an illustration in Fig. 4. Formation of these structures depends on the value of the shear rate and the duration. Higher shear rates, as reported by Chen et al. [35] and Saarikoski et al. [25], can destroy the structures. These difficulties are not encountered in dynamic measurements (small-amplitude oscillations in the linear domain), and they are considerably reduced with online rheometry using a mixing system.

Focusing now on the data provided by these measurements, the main and best known characteristic is the viscosity. Suspensions are generally shear-thinning, and this behaviour is quantified by the power-law index n and the consistency k (fit of a

Fig. 3 Illustration of "wall slip" (depletion layer) in rheometry on a 4 % vol TiO_2 suspension
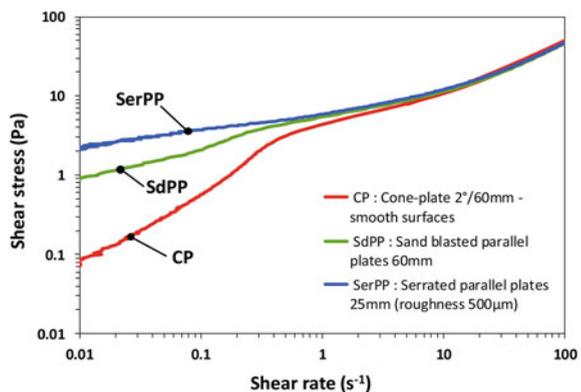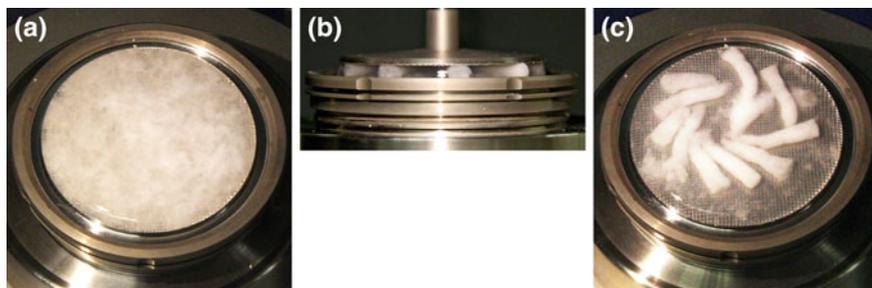
Fig. 4 An extreme case of aggregation caused by a constant unidirectional shear flow of Whatman paper suspension (1 % wdm/w), fibre length $d_{4,3}$: 310 μm, serrated parallel plates 60 mm diameter, gap: 4 mm, constant shear rate: 10 s^{-1}). **a** fluid before shearing, **b** during shearing, **c** after 15 s shearing. Note that the structures (visible on **b**) were somewhat disorganised when raising the upper plate (photo **c**), with no major changes in length or diameter

power law on the relevant region of the rheogram). This is, in practice, the only characteristic that can be obtained using a viscometer or a mixing system, together with the yield stress. The latter is evaluated from the low shear rate region of the rheogram, and several methods are used: shear stress limit when the shear rate tends to zero [17], adjustment of a Herschel-Bulkley-type model ($\tau = \tau_0 + k\dot{\gamma}^n$) [20, 23], peak torque (or shear stress) during transient flow experiments at a slow, steady rate [50] and stress ramp analysed in terms of deformation vs shear stress curve [45, 49].

With rheometers, additional rheological characterisations can be obtained with dynamic measurements. Oscillatory stress sweeps and oscillatory frequency sweeps are the most frequently used, particularly for lignocellulose suspensions [63]. Sinusoidal shear stress (or strain) gives information on elasticity (part of the strain which is phased with the shear stress) and viscosity (part of the shear rate which is phased with the shear stress). The complex modulus G* is defined as the ratio of shear stress to strain. Its real part G′ is the storage modulus (elastic contribution), and its imaginary out-phase component G″ is the loss modulus (viscous contribution). It is used to characterise the viscoelasticity or viscoplasticity of materials. Indeed, yield stress fluids behaved like solid elastic material in the linear domain (small-amplitude oscillatory), and the determination of the limit of this domain can be used to evaluate the yield stress [9, 19, 63].

2.2.2 Experimental Conditions

Lignocellulose suspensions were studied in various ranges of mass concentration varying between 0.1 and 600 gdm L^{-1} as indicated in Fig. 5. However, the majority of these studies focus on low and medium concentrations (1–200 gdm L^{-1}). Temperatures were usually between 20 and 30 °C. The pH of the suspension was generally neutral (around 7) excluding a few articles which investigated the effect of pH on the rheological behaviour (pH varies from 4 to 10) [24, 37].
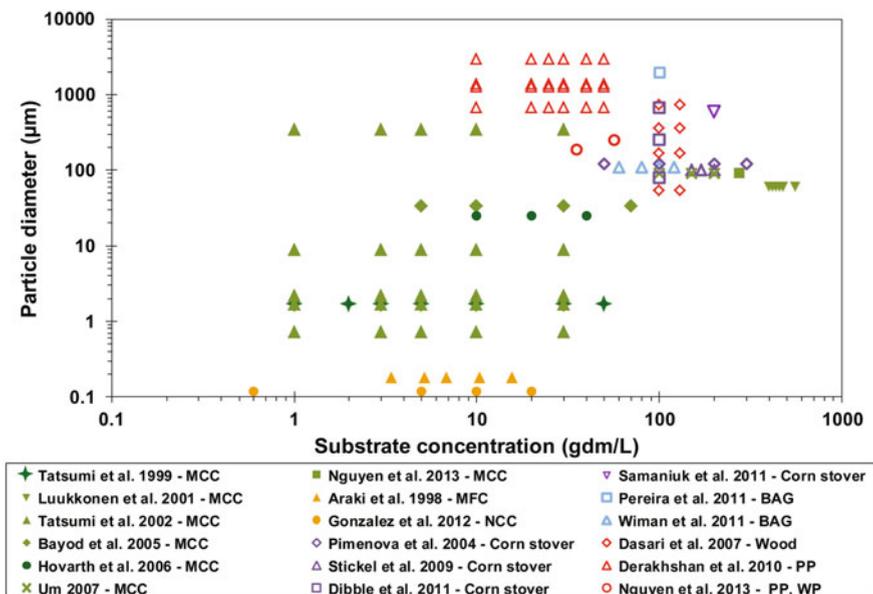
Fig. 5 Publication overview: particle diameters and substrate concentrations studied (*NCC* nanocrystalline cellulose, *MCC* microcrystalline cellulose, *MFC* microfibril cellulose, *WP* Whatman paper, *BAG* sugarcane bagasse, *PP* paper pulp)

Work scrutinising hydrolysis mechanisms was usually carried out in the range 40–55 °C for temperature, 4.8–5.0 for pH, 10–200 gdm L^{-1} for substrate concentration and 0.25–50 FPU/g cellulose for cellulase activity. Numerous studies explored two aspects: (i) hydrolysis kinetics and (ii) rheological behaviour [9, 20, 26, 27, 31–33, 38, 40–42, 51]. Items (i) and (ii) in addition to (iii) particle size evolution were investigated by Dibble et al. [32], Nguyen et al. [51] and Wiman et al. [9]. Studies of flow properties of suspensions together with hydrolysis kinetics represent half of all the articles published. This reveals the keen interest for applications concerning the valorisation of lignocellulose material by enzymatic methods, especially from 2010 on. However, simultaneous study of particle size and rheological behaviour during hydrolysis is a minor part of these studies despite the interest that this point presents.

3 Rheological Behaviour of Lignocellulose Suspensions

One critical physical parameter in lignocellulose materials is their morphology and the large aspect ratios that are encountered. They induce significant contacts among particles (fibres) even at low concentrations and bring about a strong effect on suspension rheology. An increase of aspect ratio implies increases in the suspension

viscosity [64, 65]. Not only the aspect ratio but also the particle shape influences the viscosity, as reported by Barnes et al. [66]: for 20 % volume fraction suspensions of solid particles with a limited aspect ratio (23 for the highest), and compared to a quasi-monodisperse spherical particle suspension, viscosity is 1.5-fold larger for grain suspension, 4.3-fold for plate suspension and 7.7-fold for rods. Particles with more complex shapes show greater dependence of viscosity on concentration [67, 68]. This requires a detailed knowledge of this effect, and advanced models will have to introduce critical morphological parameters to describe these evolutions.

3.1 Overview of Classical Models

3.1.1 Basic Rheological Models

The two main parameters used in rheology are the shear rate $\dot{\gamma}$ (s^{-1}), which characterises the kinematics, and the shear stress, τ (Pa or N m^{-2}), which characterises the forces.

The flow curve $\tau(\dot{\gamma})$ or equivalently the rheogram $\mu(\dot{\gamma})$, with $\mu(\dot{\gamma}) = \tau/\dot{\gamma}$, provides a complete characterisation of the rheological behaviour if the fluid is neither elastic nor thixotropic (time-dependent viscosity). Table 3 shows the main rheological behaviour and associated models. The power-law index (or flow behaviour index) n and the consistency k of the power law have a direct physical interpretation for viscosity and are present in almost all other models. They are therefore largely used as information concerning non-Newtonian viscosity.

The shear-thinning fluids (also called pseudoplastic fluids) generally have, for low and high shear rates, a Newtonian plateau (with values μ_0 and μ_∞, respectively, for viscosity). This can be modelled by using three- or four-parameter models such as Sisko, Cross, Powell–Eyring or Carreau models [69].

3.1.2 Classical Rheological Model for Suspensions

Suspension viscosity is a function of numerous parameters linked to the solid phase (particle size, shape, concentration, separated fibres/aggregates/flocs as structural units, spatial orientation of the particles/units in the fluid, etc.) and to the suspending fluid (generally a Newtonian fluid).

The classical models describing suspension viscosity concern hard sphere suspensions (Table 4). These models mainly integrate the particle volume fraction effect, and after the Einstein equation for isolated monodisperse hard spheres, a physical complexity was progressively considered with hydrodynamic and particle–particle interactions up to percolation. Then, three concentration regimes are generally considered: dilute, semi-dilute and concentrated. The first one corresponds to very small values of the volume fraction, $\varphi \rightarrow 0^+$: the average distance between particles is large compared to their radius or length. The particles can move freely

Table 3 Rheological classification of fluids and usual associated models (quantities mentioned as constant are understood as "for given temperature and pressure")

Fluids		Rheological model(s)
Perfect fluid		$\mu = 0$
Newtonian fluids		$\tau = \mu \cdot \dot{\gamma}$ with μ = const.
Time-independent non-Newtonian viscous fluids	Shear thinning: $\mu(\dot{\gamma}) \downarrow (0 < n < 1)$	Power law (or Ostwald-de Waele): $\tau = k \cdot \dot{\gamma}^n$
	Shear thickening: $\mu(\dot{\gamma}) \uparrow (n > 1)$	
	Viscoplastic (Yield stress fluid: no flow if $\tau < \tau_s$)	Bingham: $\tau = \tau_y + \mu_p \cdot \dot{\gamma}$ Herschel–Bulkley: $\tau = \tau_y + k \cdot \dot{\gamma}^n$ Casson: $\tau^{1/2} = \tau_y^{1/2} + k_c \cdot \dot{\gamma}^{1/2}$
Time-dependent non-Newtonian fluids	Thixotropic	For $\dot{\gamma}$ = Const.: $\mu(t) \downarrow$
	Anti-thixotropic	For $\dot{\gamma}$ = Const.: $\mu(t) \uparrow$
Viscoelastic fluids		Complex rheological models linking the strain rate tensor and the stress tensor. For oscillatory shear flows: $\tau = G^* \cdot \dot{\gamma}$ with $G^* = G' + iG''$ accounting for the phase shift between τ and $\dot{\gamma}$

through the suspension under the action of Brownian forces without perturbation induced by neighbouring particles but with close fluid–particle interactions (local hydrodynamic interactions). In semi-dilute suspensions, the flow perturbations created by the presence of particles will influence the movement of close particles. The overall flow pattern is affected by interactions between particles and hydrodynamic interactions. The last regime corresponds to concentrated suspensions with a lot of contacts between the particles. The viscosity of the suspension increases rapidly with volume fraction. When ϕ reaches a critical value (glassy fraction, $\phi_G \approx 0.58$ for spherical monodisperse particles), each particle is confined in a cage formed by its nearest neighbours. For volume fractions above this value, only a vibration of the particles inside the cage remains possible, and this possibility completely disappears when ϕ reaches the value of random close packing ($\phi_{RCP} = 0.64$ for monodisperse spheres) which is lower than the limit case $\phi_{FCC} = 0.74$ corresponding to face-centred cubic arrangement.

The frontier between the different concentration domains strongly depends on the nature and the intensity of interaction between the particles. For instance, a 5 % vol. suspension of neutral monodispersed spheres behaves as a dilute and Newtonian suspension, while a 1 % vol or less clay suspension is viscoplastic with a very high yield stress together with a thixotropic behaviour.

The extension of the classical models of Table 4 had successively integrated particle complexity such as morphological aspects [66] and deformable particles [69]. A higher order of complexity was incorporated by considering the flocs or aggregates currently encountered in fibre suspensions. These complex associations

Table 4 Rheological models for hard sphere suspensions [69]

Dilute	Einstein	$\mu_{\rm rel} = \frac{\mu}{\mu_s} = 1 + 2.5 \cdot \phi$
Dilute/semi-dilute	Batchelor	$\mu_{\rm rel} = \frac{\mu}{\mu_s} = 1 + 2.5 \cdot \phi + 6.2 \cdot \phi^2$
Semi-dilute/ concentrated	Krieger-Dougherty	$\mu_{\rm rel} = \frac{\mu}{\mu_s} = \left(1 - \frac{\phi}{\phi_m}\right)^{-[\mu] \cdot \phi_m}$ With $1 \le [\mu] \cdot \phi_m \le 2$ $[\mu]$: intrinsic viscosity ϕ_m: maximum volume fraction
Concentrated	Douglas-Garboczy	$\mu_{\rm rel} = \frac{\mu}{\mu_s} = K\left(1 - \frac{\phi}{\phi^*}\right)$ ϕ^*: critical volume fraction (percolation threshold)

of particles can, for instance, be modelled by introducing structural units in an effective volume fraction $\phi_{\rm eff}$ which includes the embedded fluid fraction through an average compactness [69].

3.2 Observed Rheological Behaviour

In dilute regime, Newtonian behaviour is generally observed for substrate concentrations less than 0.1 %. This critical concentration ($C^* = 0.1$ %) was proposed for both NCC [13] and MCC [17, 48]. Wu et al. [70] measured a slightly higher value for NCC ($C^* = 0.4$ %). No model of critical concentration was presented or proposed for complex matrices. This point can be explained by a lack of studies for very low concentrations which are not interesting from a lignocellulose valorisation point of view.

For substrate concentrations higher than 0.1 %, all studies report non-Newtonian behaviour, of shear-thinning or pseudoplastic type, as illustrated by Fig. 6. In this figure, these viscosities for MCC and BAG are well described by a power-law model on the studied shear rate range. Considering now the semi-dilute and concentrated regimes, a sharp increase of viscosity was observed by Nguyen et al. [51], who studied the rheology of different lignocellulose suspensions (microcrystalline cellulose, Whatman paper, paper pulp). They noticed a critical volume fraction, C^{**} (based on humid matter), that signifies the regime transition. Depending on the material complexity, this value increased from Whatman paper ($C^{**} = 3$ %) to paper pulp ($C^{**} = 9$ %) and to microcrystalline cellulose ($C^{**} = 24$ %).

Table 5 illustrates the viscosity of corn stover suspensions for a given concentration and a given shear rate. These results indicate homogeneous values although measured by different authors using different methods. These similar values could originate from the same substrate type, the same pre-treatment method (diluted acid with H_2SO_4—standard protocol from NREL) and the same range of particle diameters (100–120 µm for volume mean diameter).

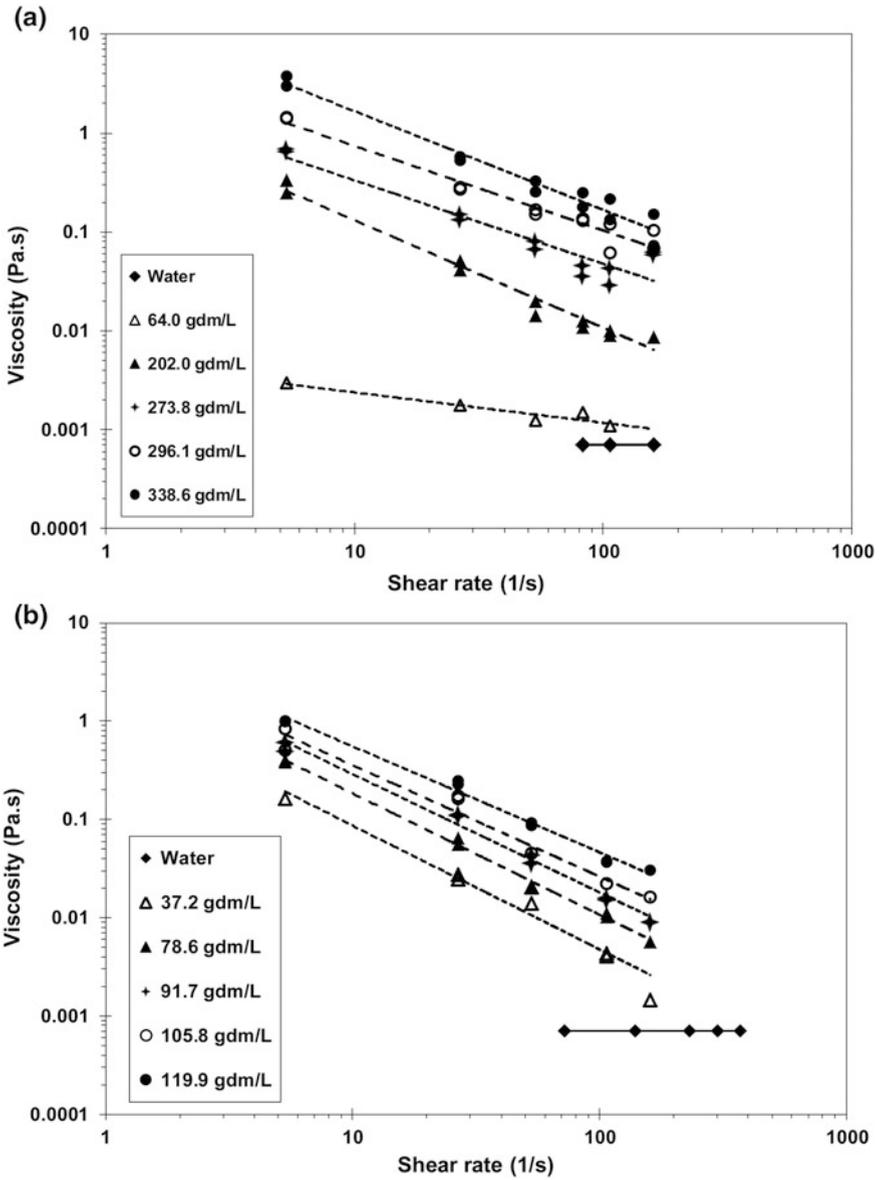**Fig. 6** Viscosity of MCC (90 μm (**a**)) and BAG (**b**) suspensions as a function of shear rate

Table 5 Comparison of viscosity for corn stover suspension at 15 % wdm/w

| Authors	Viscosity (Pa s)
Pimenova and Hanley [28]	15
Stickel et al. [29]	25
Viamajala et al. [30]	20
Dunaway et al. [31]	10

Specific values are reported for a shear rate of 10 s^{-1}

3.3 Structure and Consistency Index

As mentioned before, the flow behaviour index n and the consistency k are good indicators of viscosity. Figures 7 and 8 present data extracted from the literature for the consistency (Fig. 7) and the flow behaviour index (Fig. 8) versus particle diameter and substrate concentration. Among all the publications cited, only 25 % present these two parameters. This point evokes the difficulty for data treatment and the observation of general tendencies. Furthermore, no results were found for particle diameters less than 30 μm or larger than 600 μm and only one result for substrate concentrations above 150 gdm L^{-1} [28]. Bayod et al. [18] and Pimenova and Hanley [28] who studied, respectively, MCC and corn stover suspensions gave totally different results compared to other authors. So their results are not presented in these two figures.

Concerning the consistency index k (Fig. 7), an increase is observed when the substrate concentration and/or the particle diameter increase. With PP, for example, k increased sixfold (from 3.3 to 17.1 Pa s^n) for concentrations varying from 28 to 42 gdm L^{-1} [51]; at the same concentration of 50 gdm L^{-1}, k increased twofold (from 62 to 112 Pa s^n) for particle mean diameters varying from 670 to 2960 μm

Fig. 7 Relationship between consistency index, particle diameter and substrate concentration
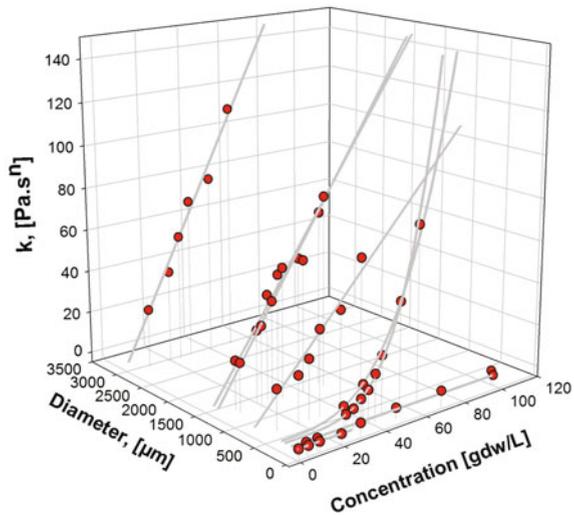
Fig. 8 Relationship between power-law index, particle diameter and substrate concentration
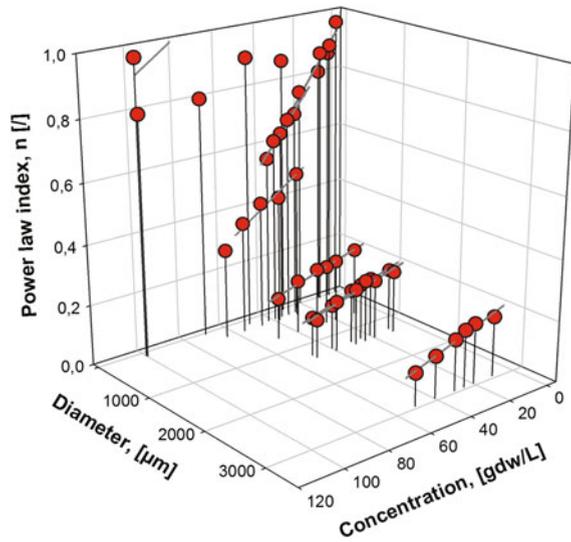
[37]. So, the effects of concentration and particle size on the consistency index are of the same order of magnitude. The relationship between these quantities can be modelled by a linear or a quadratic equation. This tendency is observed visually on graphics but also validated by Nguyen et al. [51] and Bayod et al. [18].

Now focusing on the power-law index n (Fig. 8), it decreases as the substrate concentration increases, thus underlining the fact that the non-Newtonian behaviour becomes increasingly pronounced as the concentration rises. With NCC, for example, n decreases from 0.962 to 0.75 for concentrations of 0.6 to 20 gdm L^{-1} [14], and for corn stover suspensions, n decreases from 0.91 to 0.5 for concentrations of 50 to 300 gdm L^{-1}. For particle diameters less than 1000 μm, n clearly tends to 1 as substrate concentration tends to 0, the behaviour of the suspension tends towards that of water [14, 20, 28, 51]. For particle diameters greater than 1000 μm, this tendency is not so clear on the graphs and n does not seem to tend to 1 as the concentration tends to 0. This point could be explained by a jump of power-law index for a very low concentration and could be specific to fibre morphology, aspect ratio, etc. For particle diameters greater than 1000 μm and concentrations greater than 10 gdm L^{-1}, the power-law index comes to 0.2 whatever the concentration and diameter [37]. This value can be considered as a critical value of the power-law index or simply regarded as resulting from the choice of the model (power-law model or Herschel–Bulkley model) and adjustment zone.

Considering the analogies between lignocellulose fibre and micro-organism suspensions, for cell broth, the biomass concentration and the morphology parameters clearly affect the rheological properties. Different authors tried to correlate rheological behaviour and models with various parameters (concentration, morphology, porosity) as reported in the review of Wucherpfennig et al. [11]. These authors gave a precise description of the morphological criteria of interest.

As reported by Wucherpfennig et al. [11], Tucker and Thomas [71] studied 75-h *Penicillium chrysogenum* samples and proposed that each rheological parameter (RP) could be expressed as a power-law function of the biomass concentration (C_m), the roughness and the compactness. In more detail, Riley et al. [72] used the same fungi and concluded that the average temporal value was 0.35 for a power-law index, n, with C_m ranging from 10 to 32 g L^{-1}. However, the data reported indicate that n evolves between 0.45 and 0.2. They proposed a correlation (Eq. 2) for the consistency index in relationship with biomass concentration C_m (g L^{-1}) and mean maximum dimension D_m (µm):

$$k = C_m^2 \cdot (5 \times 10^{-5} \cdot D_m - 10^{-3}) \tag{2}$$

Petersen et al. [73] proposed, using principal components analysis, a prediction of apparent viscosity, yield stress and consistency index from the size distribution and biomass concentration. Based on *Aspergillus orizae* cultures, they assumed a constant power-law index around 0.4 and reported accurate correlations between predicted and experimental parameters (k and τ_0). In a more detailed approach, Mohseni and Allen [74] investigated the influence of concentration (3–20 cdw/L) and particle morphology on the yield stress of filamentous broths of *Aspergilus niger* and *Streptomyces levoris*. They mainly focused on yield stress (ranging from 0.1 to 37 Pa) and proposed a power-law model for τ_0 as a function of biomass concentration (exponent between 2 and 3). In addition, Mohseni and Allen [74] examined different morphological parameters, reported empirical correlations and identified the most relevant morphological factors.

In view of the level of complexity of substrate suspension (cell culture broths, lignocellulose suspensions), the robustness of rheological models (consistency and power-law indexes, yield stress) has to be improved to reliably integrate the morphological parameters for filamentous and fibrous suspensions.

3.4 Yield Stress and Oscillatory Measurements

A central point in a bioprocess is to ensure correct homogenisation of the material throughout the reactor and the better control and an optimal efficiency of hydrolysis at each point. This becomes a challenge when complex rheological behaviours are encountered as is the case with lignocellulose suspensions, due to their fibrous nature, and with filamentous broth. In the dilute regime, increasing the dry matter content of a suspension is not a difficulty as it just corresponds to a reasonable increase in the viscosity which can be predicted. However, the limit for this regime depends on the nature and properties of the fibres, but whatever that may be, it is very low and extrapolation from low dry matter contents to high ones is not really feasible. But large-scale processes need higher dry matter contents to be considered as realistic from a production cost point of view. Increasing concentration is then an economical and environmental necessity but leads to complex rheological

behaviour with the appearance of yield stress. Considering hydrolysis of ligno-cellulose matrices, a balance between feed strategy (viscosity increases due to substrate concentration) and bioreaction (viscosity decreases due to fibre degradation and solubilisation) can be proposed to reach high fermentable substrate concentrations (equivalent to high dry matter content).

Lowys et al. [23], working on MFC suspensions, observed that above a critical concentration $C^* \approx 0.3$ %w/w, the viscosity rapidly increases and suspensions exhibit a viscoplastic behaviour. This signifies, inter alia, that the shear stress has to be above this critical stress to initiate flow. Consequently, the regions where the stress is not high enough are dead zones or solid-like regions which are highly damageable for transfers and thus for process efficiency [49, 75]. This viscoplastic behaviour has been widely reported in fibre suspensions [63, 76] as well as in filamentous broth [74] and has to be considered in the design of process equipment [45].

As mentioned in Sect. 2.2, yield stress fluids behave as elastic/viscoelastic materials under the threshold and their behaviour is studied using various approaches throughout the literature: by shear viscosity measurements and/or by dynamic measurements (oscillatory strain sweeps and oscillatory frequency sweeps). The latter allow the determination of the elastic modulus G′ and of the loss modulus G″ which can in turn be used to estimate a value for the yield stress. In the linear domain, the elastic modulus is generally found to be almost an order of magnitude higher than the loss modulus and independent or weakly dependent on frequency as expected for yield stress fluids.

Focusing now on pulp fibre suspensions in the domain of interest for industrial applications, increasing concentration leads from small flocs loosely connected by individual fibres to a network structure [34]. When these suspensions are submitted to small oscillatory strains, the linkages are deformed elastically. Increasing the strain amplitude and leaving the linear domain lead to inter-fibre or inter-floc failures and to a structural breakdown of the fibre network. The observed yield stress then indicates the appearance of a physical network and is associated with the strength of this network. So, yield stress and elastic modulus can also be regarded as sources of information on the fibre network.

The network properties and especially the yield stress depend on numerous parameters among which the concentration and the fibre characteristics: length, aspect ratio, pH, temperature, additives.

To illustrate the variety of these parameters, let us mention two studies. The first [23] concerns the dispersion method used to prepare the suspensions. The authors observed notably lower values for the yield stress and for the G′ and G″ modulus for mechanical stirring when compared to ultrasound dispersion. Ultrasound achieves better dispersion and is linked to a greater strength of the network. Other interesting results are reported by Dibble et al. [32] who compared two reduction size methods on the same initial material. Their results show that mechanical dispersion does not reduce the yield stress nor the enzyme digestibility of the biomass, whereas dilute acid pre-treatment leading to a similar particle size distribution leads to a decrease in yield stress and to increased digestibility.

This illustrates the complex relationship existing between the fibre properties, their implementation and the rheological behaviour of the resulting suspension. Nevertheless, some interesting results are available in the literature. Most concern the variation of the yield stress and/or the elastic modulus with concentration. Bennington et al. [45] on the basis of previous works examined a power-law relation (Eq. 3) with the mass concentration C_m:

$$\tau_y = a \cdot C_m^b \tag{3}$$

A similar relation (Eq. 4) was proposed for the elastic modulus:

$$G' = \alpha \cdot C_m^\beta \tag{4}$$

Considering the general case of suspension rheological behaviour, the appropriate parameter is rather the volume fraction of the particles. For complex systems, such as cellulose/lignocellulose fibres, the volume of fibres/flocs is not easy to define and to evaluate because the pulp fibres swell up in the presence of water to constitute a porous medium with trapped water. Because the mass concentration can be accurately determined without ambiguity or having to choose between the objects to consider, it is preferred. The drawback for this choice is the dependence of the model parameters on the nature and properties of the fibres as the relationship between volume fraction and mass fraction is not a simple one. This point was raised by Bennington et al. [45]. The correlations proposed by different authors are presented in Table 6. A large dispersion is observed for parameters a and α and so they are not reported in this table.

The most striking result from Table 6 is the large dispersion observed for the values of β and b, and the fitted parameters appear to be specific to each pulp. Nevertheless, a tendency can be noted as the values observed for the smallest

Table 6 Correlation between suspension mass fraction and yield stress and/or elastic modulus

Authors	Matrices	% d_m (w/w) (%)	$G' \propto C_m^\beta$ β parameter	$\tau_y \propto C_m^b$ b parameter
Bennington et al. [45]	PP	0.5–35	–	2.31, 2.99 and 3.56
Tatsumi et al. [17]	MCC	0.05–1	2.25	2
Dalpke and Kerekes [77]	PP		–	2 to 4
Tatsumi et al. [21]	PP	1–10	3	–
Stickel et al. [29]	CoSt	5–25	–	5.7 ± 0.5
Agoda-Tandjawa et al. [24]	MFC	0.2–3	2.58	–
Ehrhardt et al. [78]	CoSt	20–35	–	3.7 to 4.2
Knutsen and Liberatore [50]	CoSt	5–17	–	6
Wiman et al. [9]	Pre-treated spruce	4–12	6.6 ± 0.3	5 ± 0.2

particles (MCC, MFC) are smaller than those for larger fibres (pulp fibres). To explain the dispersion observed in pulp fibres, the type of fibre processing is evoked [78]. Using Eq. 3 expressed with the volume fraction ϕ, Wiman et al. [9] calculated a fractal dimension of the network.

If the existence of a correlation between yield stress or elastic modulus and mass concentration seems to be generally acknowledged, no real correlation appears between these rheological parameters and the morphological properties of the fibres, namely length and aspect ratio. This point was examined by Bennington et al. [45] who were unable to conclude as to the existence of such a correlation although theoretical considerations had led the authors to expect a squared-aspect ratio dependence. Over a reduced range of aspect ratios, Wikström et al. [47] reported a value of around 1–1.3 for this exponent.

Some studies also explored the influence of pH and temperature and generally concluded that there was a low or null influence for MFC [23, 24] as well as for pulp [47] except Ehrhardt et al. [78] who observed a decreasing yield stress as the hydrolysis temperature was increased.

As increased yield stress will lower process efficiency and raise energy costs, one can expect that a good choice of additives will modify the rheological behaviour in the right direction. This was analysed by different authors. Thus, the yield stress and/or the G′ G″ moduli are enhanced by additions of flocculent [46] or salt [23, 24]. Concerning polyelectrolyte additions, results are contrasted between Lowys et al. [23] on MFC and Horvath and Lindstrom [19] on bleached softwood kraft pulp. Testing a large range of surfactants on pre-treated corn stover, Knutsen and Liberatore [50] observed a decrease in the yield stress, while addition of protein had the opposite effect.

4 Physical Properties of Fibre and Suspension During Enzymatic Hydrolysis

Concerning the general evolution of viscosity, all studies conclude that the viscosity of suspensions decreases during enzymatic hydrolysis. This depends strongly on the nature of matrices, on the nature and the activity of the enzyme, and on the concentration of the substrates and the experimental conditions such as temperature or rotation speed. During hydrolysis, two phases can be observed for viscosity. First, a rapid decrease of viscosity is observed. Then, a steady value is maintained during a second phase. Dasari and Berson [41] studied the hydrolysis of red oak sawdust and demonstrated that viscosity decreased 10-fold after 24-h hydrolysis. For acid-pre-treated sugarcane bagasse, viscosity was reduced by 77 to 95 % after 6 h [26] and by 75 to 82 % within 10 h [27]. This decrease and the final plateau value depended on the enzyme loading [26]. For spruce pulp at 30 FPU/g cellulose, initial and final viscosities ($\mu_{initial}$/μ_{final}) were 0.24/0.028, 0.4/0.058 and 0.84/0.087 µm for concentrations of 10, 15 and 20 % (w/w), respectively [10]. This decrease of viscosity is due to mainly (i) substrate solubilisation and (ii) particle size diameter reduction. However, publications exploring

the relationship between particle size and viscosity evolution are very few. Some publications can be cited like [9, 20]. Both of these authors concluded that a roughly twofold reduction occurred in particle mean diameter for 24-h or 48-h hydrolysis.

With the goal of enabling easier comparison of viscosity variations during enzymatic hydrolysis, authors propose the use of a time $t(\mu/\mu_0 = 0.1)$ defined as the duration necessary for a 90 % reduction in viscosity. A summary of past works with different experimental conditions is presented in Fig. 9. Observing only one data series (one publication), the relationship between $t(\mu/\mu_0 = 0.1)$ and cellulase activity is clearly demonstrated: the higher the quantity of enzyme, the shorter the time $t(\mu/\mu_0 = 0.1)$. However, the tendency for all lignocellulose substrates in all experimental conditions indicates a large dispersion of points. This clearly illustrates the complexity of rheology studies in hydrolysis conditions which depend not only on enzymatic activity but also on the nature of the substrate, the bio-chemical-physico properties of the substrate and of course on experimental conditions.

Szijarto et al. [40] explored hydrolysis experiments of hydrothermally pre-treated wheat straw with purified enzymes from *Trichoderma reesei*. The results obtained at 15 % (w/w) solids revealed that endoglucanases were the key enzymes to rapidly reduce the viscosity of the lignocellulose substrate. Cellobiohydrolases only played a minor role, and xylanase had practically no effect at all on the viscosity.

Palmqvist and Liden [42] monitored the impeller torque (and hence power input) in a stirred tank reactor throughout high solid enzymatic hydrolysis (<20 % w/w) of steam-pre-treated *Arundo donax* and spruce. The decrease in torque during spruce hydrolysis was much slower than *Arundo donax* hydrolysis because of a higher
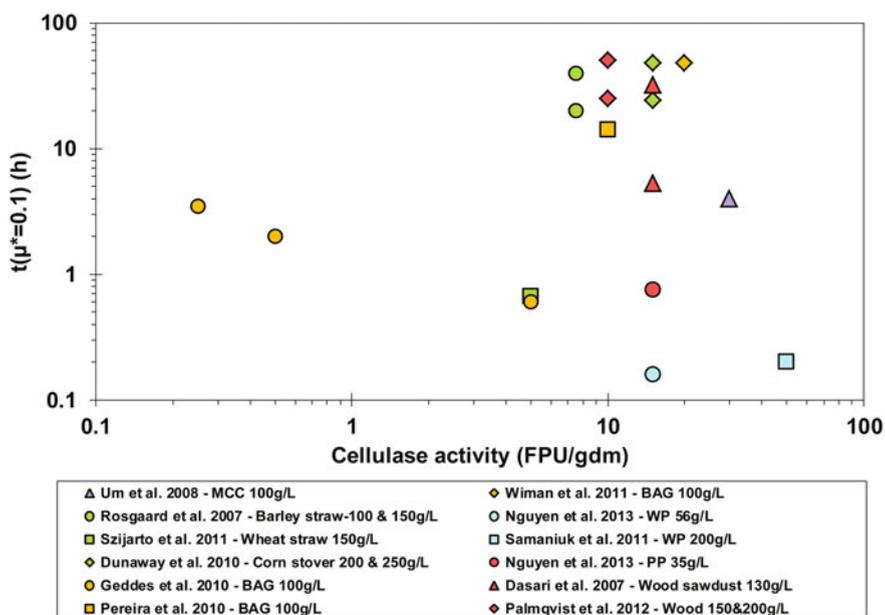

Fig. 9 Summary of $t(\mu/\mu_0 = 0.1)$ as a function of cellulase activity

amount of lignin compared to the arundo (46 and 37 %, respectively). The lignin structure is not broken down during the hydrolysis and might therefore contribute to maintaining high viscosities of the spruce material.

Concerning rheological behaviour, typical shear-thinning was confirmed during hydrolysis [27, 31, 79]. The consistency index decreases, and the power-law index increases as hydrolysis proceeds [9, 20, 31, 79]. Rosgaard et al. [79] studied the hydrolysis of steam-pre-treated barley straw and demonstrated that for a concentration of 15 % dry matter, the consistency index decreased from 16,500 to 185 Pa s^n, while the power-law index increased from 0.07 to 0.47 for 6- and 72-h hydrolysis, respectively. Dunaway et al. [31] surveyed the hydrolysis of pre-treated corn stover over a concentration range of 10–25 %. They concluded that the consistency index, k, decreases rapidly with time, with the largest rate of decrease (around 10-fold) occurring in the first 8 h.

One of the rheological parameters that were followed during enzymatic hydrolysis was the elasticity and/or the yield stress of the suspension through oscillatory measurements. However, only few articles were found on this point [9, 80, 81]. Wiman et al. [9] carried out a comprehensive rheological characterisation of dilute acid-pre-treated spruce during hydrolysis. Fillaudeau et al. [80] explored the enzymatic liquefaction and saccharification of paper pulp. Both authors confirmed that elastic modulus was always greater than viscous modulus in the initial step and during hydrolysis, confirming viscoplastic behaviour. These two modules decreased as a function of hydrolysis time around 100-fold for 48 and 100 h in spruce and paper pulp, respectively. Wiman et al. [9] demonstrated that the yield stress decreased dramatically with time. Roche et al. [81], for a 20 % w/w dilute acid-pre-treated corn stover, observed a low hydrolysis process (reduction of yield stress around 100-fold in 4 days) but obtained a single curve for yield stress versus total biomass conversion with enzyme concentrations of 5, 10 and 20 mg g^{-1}.

5 Conclusion

In a white biotechnology context, this review proposes a survey of the different methods and results concerning the rheological behaviour of lignocellulose fibre suspensions and their evolution during biocatalytic degradation. The research, initially centred on simple matrices (microcrystalline cellulose), was extended to complex substrates such as agriculture by-products and various woody substrates. The scientific literature with regard to the rheological behaviour of micro-organism suspensions (single cell up to pellet, ovoid up to mycelial morphology) constitutes a homogeneous corpus of knowledge. Nevertheless, a limited number of studies cover the rheological behaviour of lignocellulose matrices in highly concentrated suspensions and during biocatalytic degradation. A shear-thinning rheological behaviour was demonstrated with all substrates. As expected, this behaviour was conserved all along enzymatic attack, but its magnitude fell progressively. In addition, oscillatory measurements indicate a non-negligible yield stress for the

initial suspensions of several complex matrices. This yield stress rapidly decreases and then disappears within the first ten hours of enzyme attack. During hydrolysis, the evolution of viscosity is dependent on the nature of the substrate and the enzyme concentration. Conventional or adapted models accurately describe rheological behaviour of suspensions as a function of shear rate and mass or volume concentration, but the fitted parameters strongly depend on the substrate. However, a major scientific bottleneck remains in the integration of various physical parameters to establish reliable and predictive engineering models. Beyond these models taking into account shear rate and volume/mass fraction, variables such as particle equivalent diameters are sometimes included, rarely the morphological criteria and their distribution. The establishment of structured rheological models integrating biocatalytic kinetics and using high dry matter contents remains a scientific goal and is a major condition for bio-industry development. In this paper, the methods and the models related to the rheological behaviour of complex lignocellulose suspensions are similar or adapted from filamentous micro-organism suspensions including concentration, mean diameter, hyphal propensity and shear rate. By analogy, the scientific study of the rheological behaviour of lignocellulose suspensions will aim to integrate additional physical parameters such as morphological parameters (equivalent diameter, mean length, aspect ratio, specific surface area, etc.) as well as the associated distribution functions.

Future prospects depend on this state of the art. Firstly, critical concentration and hydrolysis time can be used to define a "practical feed rate" which may enable suspension viscosity to be controlled and regulated along hydrolysis depending on the release kinetics of fermentable substrates and transfer limitations. Secondly, mixed (lignocellulosic and micro-organism) suspensions should be investigated to study their rheological behaviour, define the critical and limiting parameters and integrate consolidated rheological knowledge into the models applied.

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