

A Comparison of Simple Rheological Parameters and Simulation Data for *Zymomonas mobilis* Fermentation Broths with High Substrate Loading in a 3-L Bioreactor

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Abstract Traditionally, as much as 80% or more of an ethanol fermentation broth is water that must be removed. This mixture is not only costly to separate but also produces a large aqueous stream that must then be disposed of or recycled. Integrative approaches to water reduction include increasing the biomass concentration during fermentation. In this paper, experimental results are presented for the rheological behavior of high-solids enzymatic cellulose hydrolysis and ethanol fermentation for biomass conversion using Solka Floc as the model feedstock. The experimental determination of the viscosity, shear stress, and shear rate relationships of the 10 to 20% slurry concentrations with constant enzyme concentrations are performed with a variable speed rotational viscometer (2.0 to 200 rpm) at 40 °C. The viscosities of enzymatic suspension observed were in range of 0.0418 to 0.0144, 0.233 to 0.0348, and 0.292 to 0.0447 Pa s for shear rates up to 100 reciprocal seconds at 10, 15, and 20% initial solids (w/v), respectively. Computational fluid dynamics analysis of bioreactor mixing demonstrates the change in bioreactor mixing with increasing biomass concentration. The portion-loading method is shown to be effective for processing high-solids slurries.

Keywords High-solids fermentation · Rheology · Bioreactors · Non-Newtonian fluids · Computational fluid dynamics (CFD)

Nonmenclature

τ shear stress, Pa
 τ_y yield shear stress, Pa
 n flow behavior index, dimensionless
 K consistency index constant, Pa s ^{n}
 R^2 linear regression correlation coefficient, dimensionless

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Introduction

Production of fuel ethanol from lignocellulosic biomass has the potential to reduce world dependence on petroleum while decreasing net emissions of carbon dioxide, the principal greenhouse gas [1]. There continue to be times, however, when ethanol cannot compete economically with gasoline or petroleum derivatives of fossil fuels. The opportunity therefore exists for process improvements in the conversion of biomass to fuel alcohol, resulting in more favorable production economics. High-solids loading fermentation is a process improvement aimed at increasing both the rate of fermentation and the final ethanol concentration, thereby reducing processing costs [2–4]. Positive economic advantages associated with a high-solids saccharification process over a conventional low-solids process include lower capital costs because of the reduced volume, lower operating costs because of less energy required for heating and cooling, lower downstream processing costs because of higher product concentrations, and reduced disposal and treatment costs because of lower water usage [5, 6].

Understanding the rheology of concentrated biomass slurries is important for designing equipment and predicting process performance. Specifically, the shear rate in a mixing tank is an important parameter controlling many important industrial processes. Fundamentally, shear rate affects processes involving mixing of Newtonian and non-Newtonian fluids, generating/dispersing liquid/liquid droplets, and producing fine gas bubbles for gas-to-liquid mass transfer.

Stirred tanks are typically used for the thermo-chemical fermentation. To simulate the flow of cellulosic slurries in stirred tanks, the rheological properties of these suspensions must be known. This high-solids slurry definition can be regarded as the solids region where the slurry viscosity is highly non-Newtonian at approximately 12 to 15% insoluble solids [7]. High-solids slurries such as corn stover typically range from 10 to 40% solids [8].

The primary objective of this study is to investigate the rheological behavior of high-solids cellulosic slurries, to fit an appropriate model to the experiment data and to determine the distribution of turbulent viscosity during ethanol fermentation. The results of this work will be used to investigate high-solids saccharification followed by fermentation (SFF) for biomass conversion using Solka Floc, a delignified pulp, as the model feedstock. Additionally, the results can be employed for a larger bioreactor design using computational fluid dynamics.

Materials and Methods

Materials

Solka Floc (Fiber Sale and Development, Urbana, OH), a delignified spruce pulp, was the biomass used as the raw material in this work. The composition of this material was analyzed according to National Renewable Energy Laboratory (NREL) standard procedures 001, 002, and 006. The glucan content was 88%.

Enzyme and Microorganism

Commercially produced Spezyme CP and Novozyme 188 were used for enzymatic hydrolysis. The cellulosic enzyme Spezyme CP, secreted by *Trichoderma longibrachiatum*,

formerly *Trichoderma reesei*, was procured from Genencor International (Palo Alto, CA). The enzyme had an activity of 82 GCU/g (provided by the manufacturer) and 55 IFPU/mL as determined by NREL standard procedure 006. Novozym 188 purchased from Sigma (Cat. no. G-0395) was used for cellulose hydrolysis with a volume ratio of 4 IFPU Celluclast/CBU Novozyme to alleviate end-product inhibition by cellobiose.

The recombinant *Zymomonas mobilis* strain ATCC 39679, carrying the plasmid pZB4L (designated as *Zm* 39679:pZB4L), was provided by M. Zhang (NREL, Golden, CO). Stock cultures were stored in glycerol at -70 °C.

Preculture and Inoculation Procedures

A 2.0-mL aliquot of a glycerol-preserved culture was removed from cold storage (freezer) and transferred to 200 mL of complex RM medium (yeast, KH_2PO_4 , glucose, and water) containing about 2% (w/v) glucose and 2% (w/v) xylose supplemented with tetracycline (20 mg/L) in a 500-mL Erlenmeyer flask and grown overnight at 30 °C.

Batch fermentations were inoculated by transferring around 10% (v/v) of the overnight flask culture directly to the stirred-tank bioreactor (BioFlo® 3000, New Brunswick Scientific, Edison, NJ). For the fermentations, the initial cell density was monitored spectrophotometrically to give an optical density at 600 nm in the range 0.4 to 0.5 corresponding to growth in the midexponential phase.

Saccharification Followed By Fermentation

To maximize the glucose and ethanol concentrations, substrate concentrations were employed from 10 to 20% on a dry basis, corresponding to cellulose concentrations of 8 to 17%. In several experiments employing a traditional batch enzyme reaction and fermentation of high substrate concentrations ($>10\%$), there is no visible liquid phase because of complete absorption of liquid by the biomass. In this state, no sugar and ethanol products were produced for tests between 10 and 20%. To overcome this problem, the Solka Floc was added to the reactions in three equal portions at 4-h intervals during both enzyme reaction and fermentation up to the 20% final substrate concentration. Then, the inoculum prepared as 10% by volume of the total working volume (2 L) was transferred into the reactor after enzymatic hydrolysis for 48 h. The enzyme loading was 30 FPU per gram of cellulose, supplemented by β -glucosidase to prevent product inhibition by cellobiose. The SFF experiments were operated for 96 h, initially at 50 °C for saccharification and finally at 30 °C for fermentation.

The substrate and nutrient media were autoclaved (120 °C for 20 min), but the enzyme solutions were not sterile. The Solka Floc slurry, diluted to different dry weights of solid material (10, 13, 15, and 20%), was used as a substrate.

Viscosity Measurement of High Solid Suspension

The viscosity of the suspension at different biomass concentration was measured by Modular Compact Rheometer Physica MCR 300 (Paar-Physica). Controlled shear-stress measurements were done using the concentric cylinder system with a FL 100/6W impeller at temperatures of 30, 40, and 50 °C. Samples were mixed before measurements were taken. Then, an appropriate volume was placed into the viscometer, allowing several minutes for the temperature to stabilize. The rheological measurements were performed three times for each value of biomass concentration using a fresh sample each time.

Models and Methods

Vessel Geometry

The cylindrical mixing tank simulated in this study has an ellipsoidal bottom with four equally spaced, wall-mounted baffles extending from the vessel bottom to the free surface, stirred by two centrally located six-blade Rushton turbine impellers. The tank diameter measured 0.138 m, and the baffle width was 0.008 m. The impeller diameter was 0.046 m ($D/T=3$) for both impellers. The distance between the impellers was 0.061 m. The bottom impeller center was positioned at a distance $C=T/3$ off the tank bottom. The liquid level was equal to the tank diameter, $Z/T=1.3$.

The suspension was a fermentation broth with various viscosities. The impellers were mounted on a 0.0025-m-diameter shaft rotating at 120 rpm corresponding to a range of Reynolds number of 50 to 300.

CFD Simulation Model and Tool Package

The CFD simulation employed the following setting: Type, 3D cylindrical; analysis model, multiple reference frame; turbulent model, Standard $k-\epsilon$ model; Mixsim V. 2.1.10; and FLUENT V. 6.2.20. The computational grid was defined by 570,000 unstructured, nonuniformly distributed, 182,000 nodes and tetrahedral cells. When refining the mesh, care was taken to place additional mesh points in the regions of high gradient around the blades and discharge region. Simulations were typically considered converged when the scaled residuals (continuity, X , Y , Z -velocity, k , and ϵ), normalized relative to the maximum circulating flow, fell below $6E-04$ by iteration 5,000.

Results and Discussion

Rheological Behavior of Enzymatic Hydrolysis Suspension

Figure 1a–c shows the dependence of the apparent viscosity of the enzyme hydrolysis suspension on biomass concentration. It clearly demonstrates a dramatic decrease of viscosity at the reloading point (i.e., after the initial 4 h). The experimental determination of the viscosity–shear rate and shear stress–shear rate relationships of the various formulation suspensions with different concentrations was performed with a variable speed rotational viscometer (2 to 200 rpm).

The viscosities observed were in range of 0.0418 to 0.0144, 0.233 to 0.0348, and 0.292 to 0.0447 Pa s for shear rates up to 100 reciprocal seconds and substrate concentrations of 10, 15, and 20% initial solids (w/v) measured at 50 °C, respectively. The fermentation experimental shear stress–shear rate curves are depicted in Fig. 1d.

In the fermentation, experimental results are presented for the rheological behavior of high-solids ethanol fermentation for biomass conversion using Solka Floc as the model feedstock. A recombinant strain of *Z. mobilis* 39679:pZB4L was used in SFF processes as a function of varying initial concentration of Solka Floc and constant enzyme dosage. The initial viscosities ranged from 0.024 to 0.028, 0.423 to 0.067, and 0.840 to 0.087 Pa s for shear rates up to 100 reciprocal seconds at combined temperatures (50 and 30 °C) at 10, 15, and 20% initial solids (w/v), respectively. At 120 rpm, the viscosities were 0.019, 0.078, and 0.105 Pa s at 10, 15, and 20% initial solids (w/v), respectively. One can see that the

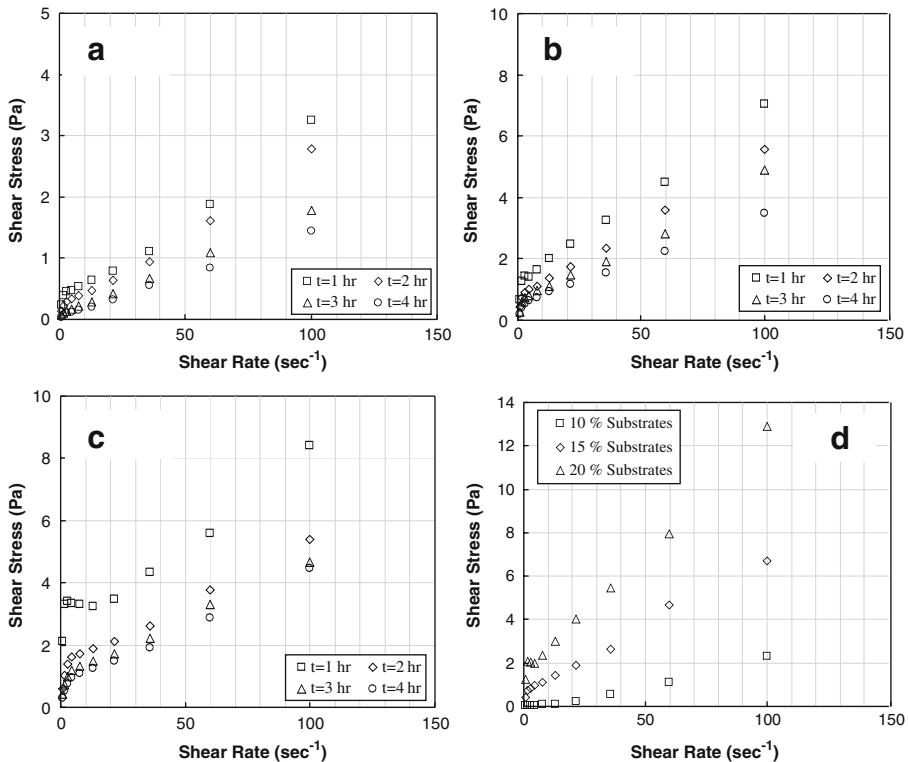


Fig. 1 Shear stress as a function of shear rate for different times during the initial 4-h enzymatic hydrolysis and fermentation. **a**, **b**, **c** 10, 15, and **c** 20% solids concentration. **a–c** Enzyme condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 50 °C, 120 rpm. **d** SFF condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 30 °C 120 rpm, *Zymomonas mobilis*, strain 39679:pZB4L

rheological behavior of suspensions of the fermentation broth significantly changes with its concentration and reaction conditions. At all concentrations, both enzymatic suspension and fermentation broths exhibit an overall pseudoplastic behavior with two Newtonian regions. At relatively high solid concentrations loaded by the portion method, constant viscosity was observed, indicating Newtonian behavior for slurries at low and high shear rates during initial enzymatic hydrolysis and SFF process.

Rheological Parameter Estimation for the Pseudoplastic Suspension

Several researchers reported viscoelastic behavior of yeast suspensions. Labuza et al. [9] reported shear-thinning behavior of baker's yeast (*S. cerevisiae*) in the range of 1 to 100 reciprocal seconds at yeast concentrations above 10.5% (w/w). The power law model was successfully applied. More recently, Mancini and Moresi [10] also measured the rheological properties of baker's yeast using different rheometers in the concentration range of 25 to 200 g dm⁻³. While the Haake rotational viscometer confirmed Labuza's results on the pseudoplastic character of yeast suspension, the dynamic stress rheometer revealed definitive Newtonian behavior. This discrepancy was attributed to the lower sensitivity of Haake viscometer in the range of viscosity tested (1.5 to 12 mPa s). Speers et al. [11] used a controlled shear-rate rheometer with a cone-and-plate system to measure viscosity of

Table 1 Determination of rheological parameter as function of time during initial 4-h enzymatic hydrolysis.

	Herschel–Bulkley model				Bingham model			Casson model				Power law		
	$\tau = \tau_y + K \dot{\gamma}^n$				$\tau = \tau_y + K \dot{\gamma}$			$\tau^{0.5} = (\tau_y)^{0.5} + n(\dot{\gamma})^{0.5}$				$\tau = K \dot{\gamma}^n$		
	τ_y (Pa)	K	n	R^2	τ_y (Pa)	n	R^2	τ_y (Pa)	n	R^2	K	n	R^2	
Reaction time (10% solid concentration)														
$t=1$ h	0.391	0.012	1.175	0.996	0.360	0.022	0.994	0.226	0.100	0.982	0.269	0.404	0.948	
$t=2$ h	0.213	0.019	1.024	0.995	0.209	0.021	0.995	0.335	0.107	0.990	0.155	0.507	0.972	
$t=3$ h	0.099	0.009	1.176	0.994	0.081	0.171	0.992	0.031	0.108	0.987	0.063	0.669	0.973	
$t=4$ h	0.065	0.005	1.294	0.995	0.044	0.014	0.988	0.013	0.105	0.983	0.037	0.766	0.972	
Reaction time (15% solid concentration)														
$t=1$ h	1.191	0.060	0.987	0.998	0.763	0.071	0.931	0.759	0.1611	0.991	0.835	0.403	0.958	
$t=2$ h	0.713	0.055	0.968	0.997	0.731	0.048	0.997	0.424	0.156	0.993	0.489	0.466	0.967	
$t=3$ h	0.577	0.044	0.975	0.996	0.589	0.040	0.996	0.338	0.144	0.992	0.399	0.476	0.969	
$t=4$ h	0.354	0.057	0.887	0.991	0.400	0.036	0.990	0.213	0.143	0.990	0.292	0.532	0.975	
Reaction time (20% solid concentration)														
$t=1$ h	2.707	0.036	1.083	0.972	2.658	0.052	0.926	1.488	0.115	0.930	2.363	0.205	0.859	
$t=2$ h	0.960	0.140	0.730	0.981	1.173	0.044	0.976	0.815	0.133	0.983	0.854	0.353	0.966	
$t=3$ h	0.600	0.154	0.693	0.987	0.827	0.041	0.979	0.535	0.138	0.989	0.589	0.408	0.978	
$t=4$ h	0.372	0.158	0.677	0.986	0.573	0.041	0.978	0.358	0.144	0.988	0.424	0.464	0.981	

Condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 50 °C, 120 rpm. Substrates were added to the reactions in portion loading during hydrolysis up to the 20% final substrate concentration.

suspensions of flocculating and nonflocculating strains of *S. cerevisiae* and *S. uvarum*. They used the Bingham model for description of viscoelastic flow behavior of cell suspension.

The normal procedure for the estimation of the model parameters for pseudoplastic fluids with a yield stress using rheological models employs nonlinear regression of the viscometric data from a concentric cylinder geometry with a numerical package, minimizing the sum of error squares. Nonlinear fits to various data with a statistics package (RHEOLPLUS) has sometimes given a best fit with negative yield values, which is meaningless. Therefore, the first point at low shear stress was not considered in the regression analysis.

Figure 1 shows shear stress curves as a function of shear rate at different time during initial enzymatic hydrolysis and fermentation processes. The yield stress values are shown

Table 2 Determination of rheological parameter as function of time during the initial SFF process.

Percent (w/v) substrates	Herschel–Bulkley model				Bingham model			Casson model			Power law		
	$\tau = \tau_y + K \dot{\gamma}^n$				$\tau = \tau_y + K \dot{\gamma}$			$\tau^{0.5} = (\tau_y)^{0.5} + n(\dot{\gamma})^{0.5}$			$\tau = K \dot{\gamma}^n$		
	τ_y (Pa)	K	n	R^2	τ_y (Pa)	n	R^2	τ_y (Pa)	n	R^2	K	n	R^2
10	0.029	0.002	1.554	0.998	0.014	0.011	0.980	0.001	0.105	0.970	0.016	0.973	0.968
15	0.306	0.177	0.756	0.985	0.467	0.068	0.978	0.256	0.201	0.988	0.425	0.549	0.979
20	0.735	0.259	0.740	0.996	1.016	0.089	0.981	0.625	0.216	0.996	0.866	0.461	0.970

Condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 120 rpm, *Zymomonas mobilis*, strain 39679:pZB4L. Temperature=30 °C. Substrates were added to the reactions in four portions during fermentation up to 20% final substrate concentration. The substrate and nutrient media were autoclaved (120 °C and 20 min).

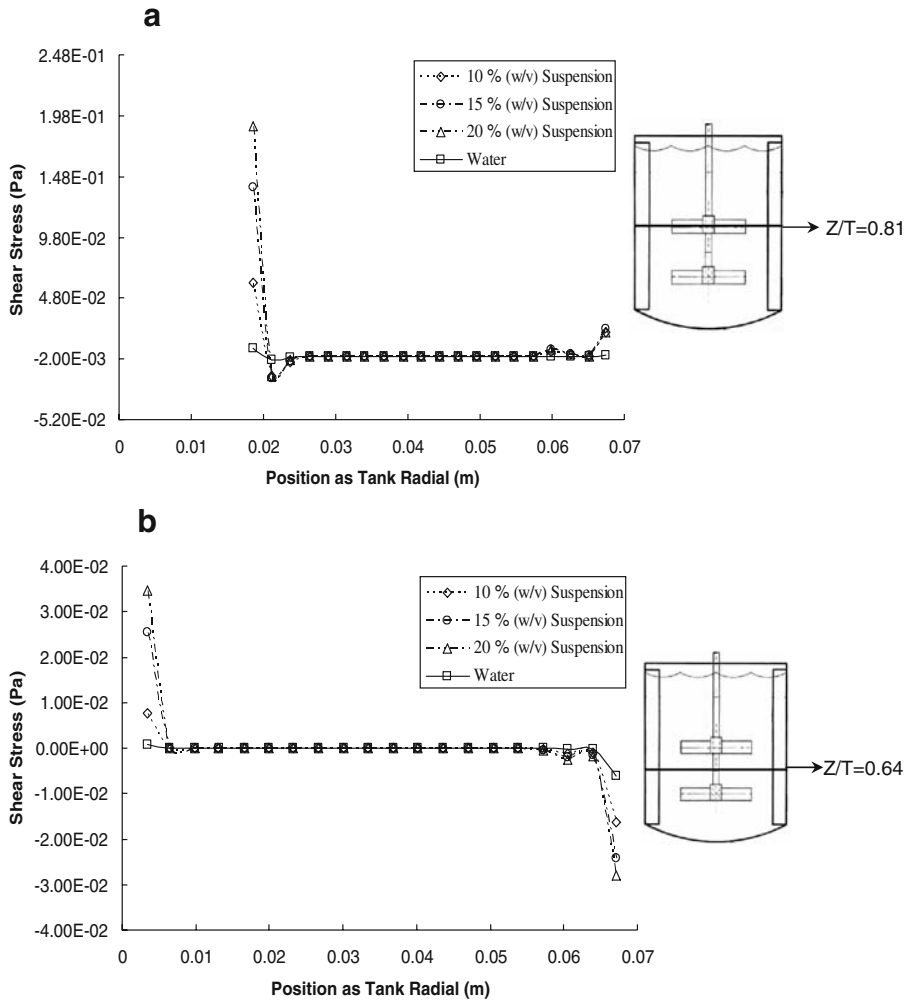


Fig. 2 Shear stress profiles as a function of tank radial position at different tank heights. **a–c** SFF condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 120 rpm, *Zyomonas mobilis*, strain 39679:pZB4L

in Tables 1 and 2. Shear stress–shear rate data of the enzymatic hydrolysis suspension and fermentation broth were tested for various rheological models (Herschel–Bulkley, Bingham, Casson, and power law models).

Four models (Herschel–Bulkley, Casson, Bingham, and power law) were used to fit the experimental data and to determine the yield stress of the slurries. Table 1 list the results obtained for the different parameters used to fit the experimental data of fermentation suspensions at the various concentrations. The Herschel–Bulkley model fits the data satisfactorily over the whole experimental range at 10 to 20% solids concentration. On the other hand, the Bingham and Casson equations are in excellent agreement with results of the enzymatic suspension and fermentation broth testing at 10 and 20%, respectively (Table 1). The results of the power law model (n and K) were compared to those power law parameters obtained with the impeller method. The Herschel–Bulkley, Bingham, and the

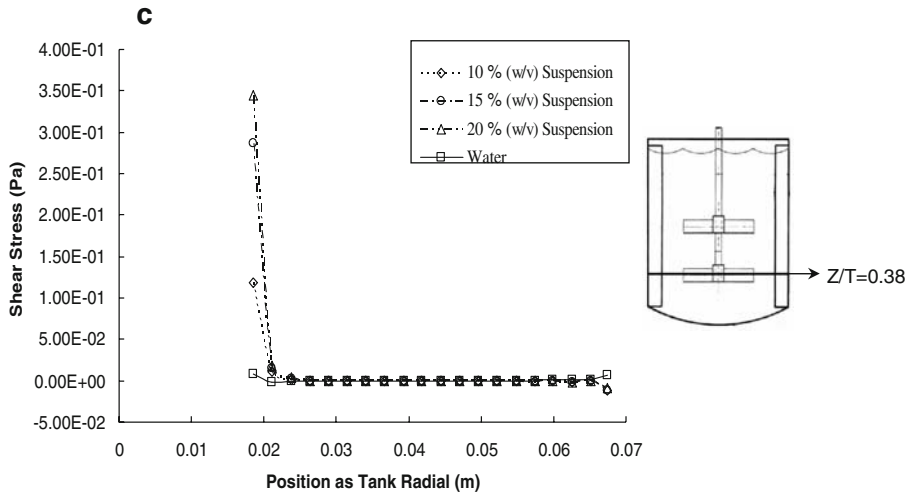


Fig. 2 (continued)

Casson models were used to compare their yield stress results to those calculated with the direct methods, the stress growth and impeller methods. Table 2 shows the parameters obtained when the experimental shear stress–shear rate data for the fermentation suspensions were fitted with all models at initial process. The correlation coefficients (R^2) between the shear rate and shear stress are from 0.994 to 0.995 for the Herschel–Bulkley model, 0.988 to 0.994 for the Bingham, 0.982 to 0.990 for the Casson model, and 0.948 to 0.972 for the power law model for enzymatic hydrolysis at 10% solids concentration (Table 1). The rheological parameters for Solka Floc suspensions were employed to determine if there was any relationship between the shear rate constant, k , and the power law index flow, n . The relationship between the shear rate constant and the index flow for fermentation broth at concentrations ranging from 10 to 20% is shown on Table 2. The yield stress obtained by the FL 100/6W impeller technique decreased significantly as the function of time and concentration during enzyme reaction and fermentation.

Simulation of Shear Stress and Turbulent Viscosity in the 3-L Mixing Tank

The viscous fermentation broth used in this project exhibited pseudoplastic rheology that is modeled quite well over a wide range of shear rates by the power law model. Consequently, the power law was used to model fluid rheology in this study for at 10, 15, 20% solids concentration. The upper and lower limits for n in this study were obtained from experiments with fermentation broth (*Z. mobilis* cultures) and ranged from 0.46 to 0.97 during the portion batch fermentation.

An understanding of the velocity flow fields is a prerequisite to understanding mixing and the key physical parameters such as shear stress, flow fluctuations, and vorticity fields. The circumferential-averaged shear stresses are plotted for the different solid concentrations as a function of tank radial position on three different panels by the z direction in Fig. 2. The fluid suspension near the impeller blade is accelerated by an imbalance of shear forces. The average maximum values determined by circumferential averaging model in the middle of tank were $0.008 \tau_{\text{avg}}$ for 10%, $0.025 \tau_{\text{avg}}$ for 15%, and $0.035 \tau_{\text{avg}}$ for 20%.

Figure 3 shows the contour of the distribution of the turbulent viscosity, modeled by three different flow behavior indexes. The maximum viscosity was found in the midpoint ($z=0.09\text{m}$) of the tank: 0.084 Pa s for 10%, 0.050 Pa s for 15%, and 0.039 Pa s for 20%. With higher values of n , the fluid viscosity was less affected by shear, and the fluid encountered a resistance that significantly impeded flow in the wall region.

Results for average shear stress and contour distributions of viscosity over the range of tank radial position in the mixing tank illustrated that the fluid viscosity was significantly reduced in the high shear stress regions. Consequently, the fluid moved rapidly through this region.

Conclusions

The rheological analysis of high-solids substrates in the bioreactor during the enzymatic hydrolysis and ethanol fermentation showed a dramatic decrease in viscosity as a function of time. Initial reaction time and specifically biomass concentration were found to affect the bioreactor hydrodynamics. Adoption of high-solids substrates loading by portion method in the 3-L bioreactor showed significant reduction in viscosity with a dramatic acceleration of net liquid flow with increasing biomass quantity. Rheological analysis revealed a direct dependence of temperature and concentration of biomass in the bioreactor on the apparent viscosity of fermentation broths.

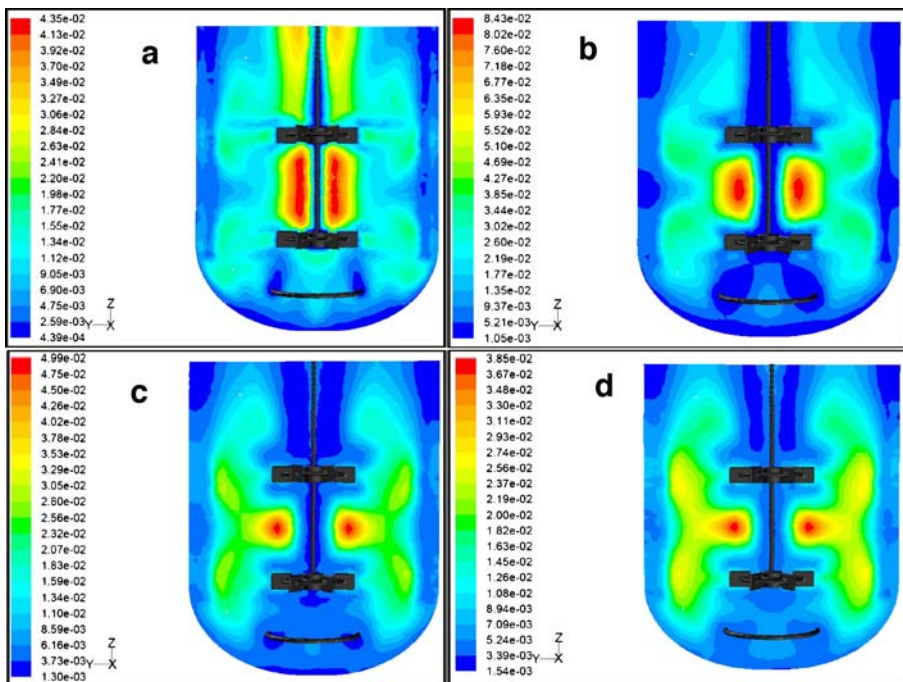


Fig. 3 Distribution of turbulent viscosity (kg/m s) in a bench scale reactor (2-L working volume). **a** Water, **b** 10, **c** 15, and **d** 20% solids concentration. **b–d** SFF condition: 30 FPU/g of glucan, pH 4.8 to 5.0, 120 rpm, *Zymomonas mobilis*, strain 39679:pZB4L

For the operation of a high-solids bioreactor for an ethanol production system, the bioreactor should operate below the critical biomass concentration to ensure efficient operation in a desirable mixing regime. The SFF process can be operated with relatively high solids loading up to a maximum solids loading of 20% w/v; however, increasing concentration affects fluid transport, mixing, and solids distribution negatively. The hydrodynamic results in the 3-L bioreactor can be used a priori or during the bioprocess to optimize operational parameters to avoid any occurrence of undesirable bioreactor stalling and to maximize the process productivity.

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