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Rheological Behavior of Schizophyllan in Fermentation System

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ABSTRACT
Schizophyllan is a neutral extracellular polysaccharide produced by the fungus *Schizophyllum commune*, consisting of a 1,3-β-D-linked backbone of glucose residues with 1,6-β-D-glucosyl side groups. The polysaccharide rheological properties have been studied in the fermentation aqueous media over the time period of 168 h. The rheology of the schizophyllan produced by *Schizophyllum commune* NRCM isolated during the 168 h fermentation is also studied by determining the consistency index, 'K' and flow behavior index, 'n' of the fermentation broth and isolated schizophyllan samples. For measurement of intrinsic viscosity, [η], the viscosity was determined at 25°C, at low polymer concentration and at low shear rate range. Schizophyllan biopolymer dispersion obeyed Power Law Model. Fermentation broth showed Newtonian behavior up to 96 h of fermentation time, beyond which the flow behavior was pseudoplastic. The molecular weight of schizophyllan was found to be 5.54×10^5 Daltons after 168 h fermentation using Mark-Houwink relation.

Key words: Schizophyllan, intrinsic viscosity, molecular weight determination, *Schizophyllum commune,* power law model

INTRODUCTION
Depending on the microbial system, some exopolysaccharide form capsules around the cells thus becoming the part of the cell wall while others form slimes which accumulate outside the cell wall and have the ability to diffuse away into the liquid phase during the course of fermentation. As a result of production of exopolysaccharides, the viscosity and the rheology of the fermentation broth may undergo profound changes. It generally starts out as a low viscosity Newtonian fluid and ends up as a highly viscous non-Newtonian fluid. These properties widely depend on the nature and the type of molecular arrangement of polysaccharide, its chemical structure, conformations and forces acting between them. Viscosity is an attribute of rheological properties. The rheological properties of aqueous solution of exopolysaccharides are of paramount importance to the mixing; oxygen mass transfer and heat transfer characteristics of polysaccharide fermentations. Non-Newtonian broths such as those with high concentrations of biomass or those producing extracellular polysaccharides such as xanthan and pullulan, exhibit pseudoplastic behavior (Iran, 2009).

In aerobic fermentations, such as curdlan, of all the nutrients supplied, oxygen is the least soluble and frequently becomes the limiting nutrient. Since curdlan is insoluble in water, the fermentation broth is of relatively low viscosity and there is little resistance to oxygen transfer from gas to the liquid. However, a layer of insoluble exopolysaccharides surrounding the cell offers the resistance to oxygen transfer from liquid into the cell and therefore a high dissolved oxygen concentration is required for maximal productivity (Lawford and Rousseau, 1991). Oxygen limitation to the submerged culture of *Schizophyllum commune* has been confirmed to stimulate the formation of Schizophyllan in a stirred tank bioreactor (Shu *et al*., 2005). Rau *et al*. (1992) observed an improvement of exopolysaccharides production when cultures of *Schizophyllum commune* were grown under oxygen limitation. One explanation for these observations may be that in the case of glucans, exopolysaccharides synthesis follows the growth phase.

Schizophyllan is an extracellular polysaccharide produced by the fungus *Schizophyllum commune*; it consists of linearly linked β-1, 3-D-glucose residues with one β-1, 6-D-glucose side chain for every three main chain residues. It was found from viscosity and sedimentation equilibrium measurements; that this polysaccharide shows very characteristic solubility behavior. Thus it disperses in water as a rod like trimer with a triple-helical structure while it disperses in dimethyl sulfoxide (Me_2SO) as a single randomly coiled chain. The conformational transitions of schizophyllan have been also studied in aqueous alkaline solutions by high-sensitivity Differential Scanning Calorimetry (DSC) and optical rotation measurements (Kitamura *et al*., 1996). Schizophyllan and scleroglucan gels melt between 5 and 20°C, depending on the glucose concentration in the solvent. It suggested that schizophyllan gels are organized in bundles (consisting of many individual polymers) with strong intra-bundle attractions and weak inter-bundle forces (Bot *et al*., 2001). In another study, aqueous solution of Schizophyllan-D-Sorbitol formed a thermo reversible gel upon cooling. The gelation was considered to be induced by the transition from Schizophyllan triple helix II to I which leads to a three-dimensional network constituted by the extremely entangled and stiff Schizophyllan triple helices I (Fang and Nishinari, 2004).

The generalized relationship between the shear stress and shear rate for a fluid is given by Eq. 1 (Margaritis and Pace, 1985):

$$\tau = \tau_o + K\,(\gamma)^n \tag{1}$$

where, 'τ' is the shear stress applied on the fluid (FL^{-2}), 'τ_o' is the yield stress, 'K' is the constant index ($FL^{-2}\,T^n$), 'n' is the dimensionless flow index and 'γ' is the fluid velocity gradient, also referred to as the shear rate (T^{-1}).

Depending upon the required accuracy and of fitting, given experimental shear stress versus shear rate data, the proper rheological classification of exopolysaccharides solution may be at times somewhat difficult. This is particularly true when one tries to fit the experimental rheological data of a given exopolysaccharides solution system over a wide range of shear rates.

The molecular weight of schizophyllan has been reported to be estimated by various techniques as size exclusion chromatography coupled with low angle laser scanning and by using intrinsic viscosity, [η].

Intrinsic viscosity is the characteristic property of an isolated polymer molecule in given solvent and represents measurement of its hydrodynamic volume. Intrinsic viscosity, [η] is related to the conformation in solution and the molecular weight of macromolecule. It depends primarily on molecular weight, chain rigidity, electrical charge, as well as the solvent quality. For measurement of intrinsic viscosity, [η], the viscosity is determined at 25°C, at low polymer concentration and at low shear rate range. The intrinsic viscosity [η] can be calculated by using following formula:

$$[\eta] = \lim_{C\to o}\left[\frac{\eta solution\text{-}\eta solvent}{\eta solvent^{\cdot c}}\right]$$

where, c is the concentration of the polymer.

Viscosity results as a function of polymer concentration are expressed conveniently by means of Huggins equation (Huggins, 1992):

$$\frac{\eta solution}{\eta solvent^{\cdot c}} - 1 = [\eta]c + k'[n]^2 c^2$$

where, k' is the Huggins constant and is representative of the interaction of the polymer with its solvent.

Intrinsic viscosity can be determined by using plot of polymer concentration vs. specific viscosity where concentration tends to 0. The 'Y' intercept is taken as value of intrinsic viscosity [η]. The results of viscometric analyses was correlated by using Mark-Houwink relation:

$$[\eta] = K_s\, M_w{}^a$$

where, [η] is intrinsic viscosity, M_w is average molecular weight and K_s is the Mark-Houwink relation constant traditionally deduced from measurements of 'M_w' and 'a', the Mark-Houwink exponent, relates the power law dependence of molecular weight of the intrinsic viscosity. It is related to the shape of the molecule (Kulicke *et al*., 1997). An exponent value 'a' of 1.8 is the theoretical prediction for a rigid rod conformation. In case of high molecular weight polymers which behave more like semi-rigid chain, a lower 'a' value of 1.1 has been suggested. In case of schizophyllan aqueous solutions, a value of 1.26 was applied. The value of K_s was calculated according to previous results for the intrinsic viscosity [η] and the molecular weight, M_w, determined by light scattering for schizophyllan. This value was 3.39×10^{-5} ([η] in (mL g^{-1})).

The objective of this part of work is to study the rheological behavior of the fermentation broth with respect to time. The rheology of the schizophyllan produced by *Schizophyllum commune* NRCM isolated during the 168 h fermentation is also studied by determining the consistency index, 'K' and flow behavior index, 'n' of the fermentation broth and isolated schizophyllan samples.

MATERIALS AND METHODS

The study was carried out in the month of November 2005.

Materials: Media components: Sucrose, beef extract, potassium dihydrogen phosphate, magnesium sulphate heptahydrate was procured from Himedia, Mumbai. UDP-glucose, isopropyl alcohol was procured from S. D. Fine Chemicals Ltd, Mumbai.

Microorganism: Strains of *Schizophyllum commune* NRCM was isolated from NRCM, Solan, Himachal Pradesh, India.

Methods: Schizophyllan production is carried out in two stages. In the first stage, cells are grown in the seed culture; and in the second, seed culture are inoculated into the fermentation medium for schizophyllan production (Kumaria *et al*., 2008).

Schizophyllan was isolated from the broth by the procedure described by Rau *et al*. (1992).

Rheological behavior of the fermentation broth: In order to study the changes in the viscosity of the fermentation broth during the course of fermentation for schizophyllan production, samples were withdrawn at every 24 h during the course of 168 h fermentation and the viscosities for the same were determined using Haake Rotational Rheometer RT10 (Cone and Plate) at different shear rates. Rheological properties were studied by substituting the viscosity data into the Power law model $\eta = K\gamma^{n-1}$ where, η is the apparent viscosity (mPa.s) and γ is the shear rate (sec^{-1}) and then calculating the flow behavior index 'n' and the consistency index 'K' of the broth.

Rheological study of schizophyllan solution: Schizophyllan samples were isolated as per the procedure described above from the fermentation broth every 24 from 72 h (up to 168 h). 1% solution in water was prepared and used for the study. A Haake Rotoviscometer (VT550) with a sensor system NV (cup) and NV (rotor) was used for this purpose. The consistency index 'K' and the flow behavior index 'n' were determined from the power law model given by the equation $\eta = K\gamma^{n-1}$, where η is the apparent viscosity (mPa.s) and γ is the shear rate (sec^{-1}). The value of 'n' was obtained from the slope of the log-log plot of viscosity versus shear rates. The value of 'K' was calculated from intercept of the same graph.

Molecular weight determination of samples at different stages of fermentation: The molecular weight is a characteristic property of any polysaccharide. It keeps changing with the fermentation time. Molecular weight of the schizophyllan sample isolated after 168 h fermentation was determined by using intrinsic viscosity data and Mark-Houwink equation.

Schizophyllan sample was isolated from fermentation broth at the end of fermentation i.e., 168 h. Schizophyllan solutions at 0.1-0.9% were prepared and the viscosity was measured at lowest shear rate of 27.05 S^{-1}, by using Haake Rotoviscometer (VT550) with a sensor system NV (cup) and NV (rotor). Specific viscosity was calculated as:

$$\frac{\eta solution - \eta solvent}{\eta solvent^{\cdot c}}$$

Intrinsic viscosity was calculated from the plot of specific viscosity vs. concentration as a Y intercept. The molecular weight was calculated by using the Mark- Houwink equation given as:

$$[\eta] = K_s M_w^{a}$$

where, $[\eta]$ is intrinsic viscosity; M_w is average molecular weight and K_s is the Mark-Houwink relation constant traditionally deduced from measurements of 'M_w' and 'a', the Mark-Houwink exponent, relates the power law dependence of molecular weight of the intrinsic viscosity. It is related to the shape of the molecule. In case of schizophyllan aqueous solutions, a = 1.26 and Ks = 3.39×10^{-5} was applied.

RESULTS

Rheology of fermentation broth: Table 1 documents the results for changes in the viscosity of the fermentation broth during the course of fermentation for schizophyllan production at different shear rates. A decrease in the viscosity with increasing shear rates for samples after 72 h indicate

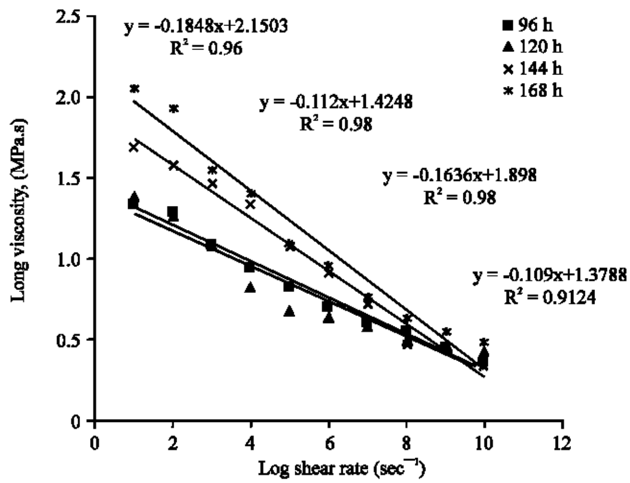

Fig. 1: Log-Log plot of viscosity of the fermentation broth vs. shear rate after 96, 120, 144 and 168 h of fermentation

Table 1: Effect of shear rate on the viscosity[a] of the fermentation broth during the course of fermentation for schizophyllan production by *S. commune* NRCM

	Apparent viscosity[a] (mPa.s)			
Shear rates γ (sec^{-1})	96 h	120 h	144 h	168 h
27.05	21.4	24.10	48.30	111.00
44.90	16.9	18.20	37.10	84.00
75.19	12.0	11.90	28.90	34.70
125.5	8.69	6.69	21.40	25.40
245.0	6.51	4.73	11.90	12.20
349.4	4.98	4.29	7.99	8.82
583.1	4.04	3.73	5.16	5.66
971.6	3.54	3.11	2.92	4.18
1610.0	2.75	2.83	2.80	3.47
2705	2.25	2.66	2.17	3.00

[a]Values are mean of two determinations

Table 2: Flow behavior index, 'n' and consistency index, 'K' of the fermentation broth after various stages of fermentation

Time (h)	Flow behavior index 'n'	Consistency index 'K' (mPa.s)
48	1.09	12.20
72	1.02	18.90
96	0.98	25.50
120	0.89	58.00
144	0.84	77.73
168	0.81	141.30

their shear thinning behavior. Up to 72 h, the broth showed characteristics of the Newtonian fluids. Fig. 1 shows the log-log plot of viscosity of the fermentation broth *vs.* shear rate at 96, 120, 144 and 168 h of fermentation. The flow behavior index, 'n' was obtained as from the slope and the consistency index 'K' was obtained from the intercept. These data are compiled in Table 1. After 72 h, flow behavior index 'n' was <1 indicating the pseudoplastic behavior of the fermentation broth

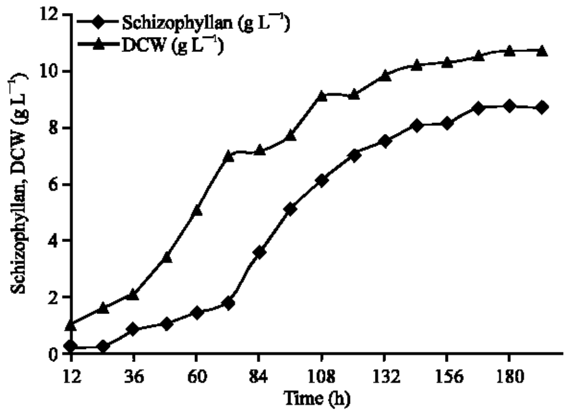

Fig. 2: Growth profile of *S. commune* NRCM

and consistency index 'K' gave increasing values (Table 2). The two major factors contributing to the viscosity of the fermentation broth are the biomass concentration and the concentration of the schizophyllan. The possible reason for the observed results of the variation in 'n' and 'K' can be explained with reference to the growth curve of *Schizophyllum commune* NRCM depicted in Fig. 2. It is clear from Fig. 2 that the cells were in the lag phase up to 72 h indicating low viscosity of the broth. The values for 'n' and 'K' showed that broth behavior is like Newtonian fluids during this period. The cell growth as well as schizophyllan production started after 72 h and reached the log phase between 72-168 h during which the viscosity of the broth was quite high. During log phase, 'n' varied in a narrow range, whereas 'K' showed an increase due to an increase in the schizophyllan production. Fermentation broth showed Newtonian behavior up to 96 h of fermentation time, beyond which the flow behaviour was pseudoplastic. This was in accordance to the growth curve of the cells and production profile of the polymer.

Rheology of schizophyllan: Schizophyllan is the homopolysaccharide of glucose. The formation of the polysaccharide in the fermentation broth can be monitored by determining the changes in the viscosity of the schizophyllan sample isolated at various stages of fermentation. The viscosity of schizophyllan, like all polymers, is related to its molecular weight. Further determining the flow behavior index 'n' and the consistency index 'K' of the schizophyllan solution in water can give an idea about the viscosity and molecular weight of the product formed at the end of the fermentation. Table 3 documents the viscosity data for schizophyllan samples that were withdrawn from the fermentation broth at various time intervals during the entire course of fermentation.

1% schizophyllan solutions were used for the study. A decrease in the viscosity with increasing shear rates for all samples indicates their shear thinning behavior. Figure 3 shows the Log-Log plot of the viscosity *vs.* shear rate of schizophyllan isolated during course of fermentation. The consistency index 'K' and the flow behavior index 'n', calculated from these graphs obeyed the power law model for pseudoplastic fluids (Table 4). The 'K' values of schizophyllan samples isolated from the fermentation broths increased with increasing times of fermentation up to 168 h and after that there was decrease in consistency index which may be due to the degradation of the schizophyllan molecule by the glucanases produced after 72 h.

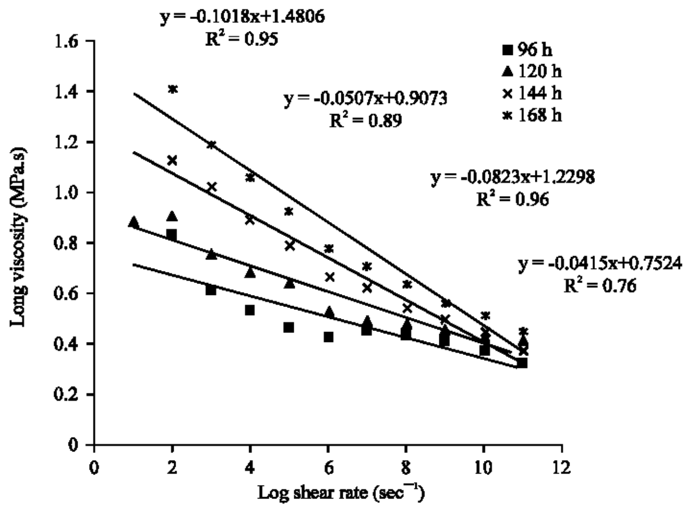

Fig. 3: Log-Log plot of the viscosity vs. shear rate of schizophyllan isolated during course of fermentation

Table 3: Effect of shear rate on the viscosity of the 1% schizophyllan, isolated during the course of fermentation for schizophyllan production by *S. commune* NRCM

	Apparent viscosity[a] (mPa.s)			
Shear rates γ (sec^{-1})	96 h	120 h	144 h	168 h
27.05	6.70	8.05	13.40	25.40
44.90	4.04	5.65	10.50	15.30
75.19	3.37	4.82	7.72	11.30
125.5	2.89	4.33	6.07	8.38
245.0	2.66	3.40	4.59	5.92
349.4	2.80	3.10	4.15	5.08
583.1	2.69	3.04	3.48	4.29
971.6	2.57	2.87	3.13	3.63
1610.0	2.34	2.66	2.78	3.22
2705	2.10	2.58	2.35	2.77

[a]Values are mean of two determinations

Table 4: Flow behavior index 'n' and consistency index 'K' of schizophyllan samples isolated at various stages of fermentation

Time (h)	Flow behavior index 'n'	Consistency index, 'K' (mPa.s)
48	1.03	2.75
72	1.00	3.42
96	0.98	5.65
120	0.96	8.07
144	0.92	16.97
168	0.90	30.04

Figure 4 documents the specific viscosity as a function of concentration of the schizophyllan samples isolated at the end of fermentation, from which the intrinsic viscosity for sample was calculated as the Y intercept. The molecular weight of the schizophyllan sample isolated at the end of fermentation was calculated from the intrinsic viscosity as per the Mark-Houwink equation. The

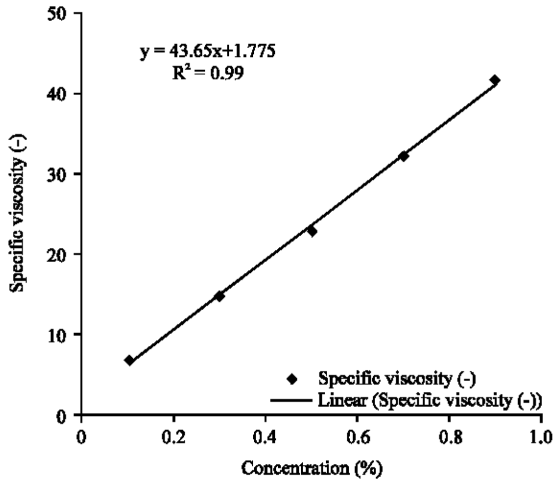

Fig. 4: Effect of concentration on specific viscosity, of schizophyllan sample isolated at 168 h

Mark-Houwink constant[a] , relates the power law dependence of molecular weight of the intrinsic viscosity. It is related to the shape of the molecule. In case of schizophyllan aqueous solutions, a =1.26 and Ks = 3. 39×10^{-5} was applied. Fermentation broth showed Newtonian behavior up to 96 h of fermentation time, beyond which the flow behaviour was pseudoplastic. This was in accordance to the growth curve of the cells and production profile of the polymer.

Schizophyllan dispersion obeyed Power Law Model. Molecular weight of schizophyllan was found to be 5.54 ×10^{5} Daltons after 168 h fermentation.

DISCUSSION

Aqueous solutions of Schizophyllan show thixotropic, pseudoplastic and viscoelastic behavior. The molecular weight ranges from 6-12×10^{6} g moL^{-1} (Rau, 1999, 2004). Upto molecular weight of 5×10^{5} g moL^{-1} the triple helix remains rigid underlined by a Mark Houwink constant[a] 0.49 (random coil= 0.5, rigid rod= 2) (Rau *et al*., 1992b).

The viscosity decreased with increasing shear rate and shear viscosity depend on concentration of Schizophyllan. Comparison of different concentration of Schizophyllan (>5 gL^{-1}) yielded viscosities >10 Pa s at low shear rate (0.3 sec^{-1}). The mean value of 0.3 g L^{-1} Schizophyllan solution at low shear rate (0.3 sec^{-1}) varied between 50-150 mPa.s (Rau, 2005). In this study the fermented broth of Schizophyllan (>7 g L^{-1}) was studied and result showed viscosities in the range of 21.4-111 mPa.s at applied shear rate of 27.5 sec^{-1} . The dried Schizophyllan sample solution (1%) yielded viscosities 21.05 mPa.s at shear rate of 27.05 sec^{-1} and the mean value of 1% Polymer solution varied between 6.7-21.05 mPa.s.

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