



Rheological properties of aqueous solution of new exopolysaccharide secreted by a deep-sea mesophilic bacterium

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ARTICLE INFO

Article history:

Received 18 August 2010

Received in revised form

29 November 2010

Accepted 24 December 2010

Available online 8 January 2011

Keywords:

Rheology

Oscillatory test

Thixotropy

Creep and recovery test

Critical concentration

SM-A87 EPS

ABSTRACT

The rheological properties, such as shear flow behavior, thixotropy and viscoelasticity, of aqueous solution of a new type of exopolysaccharide (SM-A87 EPS) secreted by a deep-sea mesophilic bacterium were investigated using shear flow and dynamic rheological measurements. For the SM-A87 EPS solutions, the overlapping concentration C^* and crossover concentration C^{**} were confirmed to be 0.95 g/L and 4.99 g/L respectively by the concentration-dependences of rheological parameters, such as equilibrium viscosity, thixotropic strength, static and dynamic stress, critical shear rate, and storage modulus in the linear viscoelastic region besides the zero-shear viscosity of solutions. At concentrations higher than C^* , the solutions exhibited a static stress, a dominant elastic behavior and a stronger absolute positive thixotropic strength. Otherwise, at concentrations lower than C^* , no static stress, a dominant viscous behavior and a weaker absolute positive thixotropic strength were exhibited. The SM-A87 EPS solutions may be used as enhanced oil recovery system.

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1. Introduction

The family *Flavobacteriaceae*, belonging to the phylum *Bacteroidetes* [formerly *Cytophaga-Flavobacterium-Bacteroides*, (CFB)], includes a number of marine bacteria. Recently, a novel genus of the family *Flavobacteriaceae*, the mesophilic bacterium *Wangia profunda* SM-A87 (SM-A87), was isolated on marine agar 2216 medium (Difco) from deep-sea sediment samples taken from near the southern Okinawa Trough at a water depth of 1245 m using core sampler (Qin et al., 2007). In order to survive in the deep-sea nutrient-scarce environment, SM-A87 secretes large quantities of highly viscous exopolysaccharides (EPS) to concentrate proteinaceous particles and metal ions as a growth and energy source. The low-nutrient consuming and high yielding properties of the bacterium indicate that SM-A87 EPS might be a low-cost producer (Zhou, Wang, Shen, Hou, & Zhang, 2009). The SM-A87 EPS may be chemically considered as an anionic polyelectrolyte, expected to be used as food additives, sorbents (Zhou et al., 2009), emulsifier, stabilizer, thickener, enhanced oil recovery system and so on. Understanding

the rheological behavior of SM-A87 EPS solution is of applied and fundamental importance.

Zero-shear viscosity, yield stress, thixotropy and viscoelasticity are the basic rheological properties which have received a lot of attention by many researchers (Barnes, 1999; Bradna, Quadrat, & Dupuis, 1995; Choi & Han, 2003; Chronakis & Alexandridis, 2001; Hou, Sun, Han, Zhang, & Wang, 1998; Lund, Lauten, Nyström, & Lindman, 2001; Moller, Mewis, & Bonn, 2006; Quadrat, 1985; Stadler et al., 2006; Takigawa, Kadoya, Miki, Yamamoto, & Masuda, 2006; Yang, Bick, Shandalov, Brenner, & Oron, 2009). Zero-shear viscosity (η_0) is the viscosity measured in shear deformation when the shear rate is approaching to zero (Biro, Gandhi, & Amirkhanian, 2009). Yield stress (σ_0) is another important rheological parameter for fluids. It is theoretically defined as the stress at which the fluid first starts moving when the applied stress increases or first stops moving when decreasing the applied stress (Barnes, 1999; Moller et al., 2006). The zero-shear viscosity and yield stress of polymer solutions could be determined by using many methods including equilibrium shear flow, creep and oscillatory shear (or sweep) measurements. The zero-shear viscosity and yield stress of polymer solutions were found to be different values depending on the measurement method and the experimental procedure (Barnes, 1999; Biro et al., 2009; Moller et al., 2006). It is possible that there is no way to measure the absolute values of them in the sense that they could be merely theoretical concepts (Barnes,

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1999). However, the existence of problems cannot cover their great industrial applications, just as Nguyen and Boger (Barnes & Walters, 1985; Blair, 1933) put about yield stress: “Despite the controversial concept of the yield stress as a true material property. . . , there is generally acceptance of its practical usefulness in engineering design and operation of processes where handling and transport of industrial suspensions are involved.” One method used for such applications is to work with two yield stresses, *i.e.*, static yield stress and dynamic yield stress (Moller et al., 2006; Papanagopoulos, Pierri, & Dondos, 1998). Static yield stress means the yield stress measured in an undisturbed sample while dynamic yield stress is the yield stress of a completely devastated sample, often determined from extrapolation of the equilibrium flow curve (Yang et al., 2009).

The thixotropy of fluids is a time-dependent rheological property. Hitherto, three types of thixotropy have been observed: “positive thixotropy”, *i.e.*, time-dependent shear-thinning phenomenon of fluids such as drilling mud, paint, coating, etc.; “negative thixotropy”, *i.e.*, time-dependent shear-thickening phenomenon of fluids mainly including polymer solutions (Bradna & Quadrat, 1984; Bradna et al., 1995; Quadrat, 1985); “complex thixotropy”, *i.e.*, the phenomenon that a given fluid may show, early or late, positively thixotropic character and negatively thixotropic character, or vice versa (Hou et al., 1998). In general, three methods can be used to investigate the thixotropic properties of a fluid: monitoring the change of viscosity (or stress) with time at a given shear rate (Abu-Jdayil, Al-Malah, & Asoud, 2002), monitoring the recovery of viscosity (or stress) with time at a given low shear rate after intense shear (Hou et al., 1998; Li et al., 2003), and the hysteresis loop (Dolz, Bbugaj, Pellicer, Hernández, & Górecki, 1997). The so-called hysteresis loop is a diagram of shear stress as a function of shear rate, which is usually obtained through a three-step experiment. First, the shear rate is linearly increased with a given ramp from zero to a prefixed maximum value. This is followed by a keeping at the maximum shear rate for a given time (peak hold). Thereafter, the shear rate is reversely decreased at the same ramp from the maximum value to zero. During these steps the corresponding changes of shear stress vs. shear rate are automatically measured in a computer-based rheometer and up-curve and down-curve is obtained, respectively. It is well known that the hysteresis loop of up-curve above down-curve means positive thixotropy, and that of up-curve below down-curve means negative thixotropy, while the hysteresis loop with a crossover point means complex thixotropy (Dai, Hou, Duan, & Ni, 2007). The hysteresis loop area enclosed between up-curve and down-curve may be considered as an estimation of the degree of thixotropy. It is generally admitted that the greater the hysteresis area, the stronger the thixotropic property.

The concentration dependence of rheological properties of fluids is of critical importance for their industrial applications. Adam, Delsanti, and Jannink (1976) presented three concentration domains for polymer solution: dilute, semidilute, and concentrated solutions. The properties of polymer solutions tend to change rather abruptly in crossing over both from dilute to semidilute regimes and from semidilute to concentrated regimes (Adam et al., 1976). In dilute polymer solutions, the molecules are separated and the intrinsic properties of the individual molecule almost determine their physical properties. When the concentration increases gradually to a certain value C^* (overlapping concentration), the molecules start to touch each other and entangle permanently. So C^* is regarded as a critical concentration which separates the dilute and semidilute regimes. At higher concentrations, intermolecular entanglements strengthen and at another critical concentration (C^{**}), entangled polymer molecules gain a uniform polymer segment density. So C^{**} is regarded as the boundary of semidilute and concentrated regimes (Roots & Nyström, 1979; Ying & Chu, 1987).

In general, the first critical concentration C^* and the second critical concentration C^{**} of polymer solutions were determined by using the concentration dependence of zero-shear viscosity (Benchabane & Bekkour, 2008). Rodd, Dunstan, and Boger (2000) reported that dynamic light scattering method could be used to determine both the critical concentrations. It is also suggested that the second critical concentration could be considered as the critical concentration at which the viscoelastic properties do appear in polymer solutions according to dynamic (creep and frequency-sweep) measurement results (Benchabane & Bekkour, 2008; Ebagninin, Benchabane, & Bekkour, 2009).

In order to get a much refined insight into the rheological behavior of SM-A87 EPS solution, the zero-shear viscosity, yield stress, thixotropy and viscoelasticity of the solution were examined in this work, and the critical concentrations, C^* and C^{**} , were determined based on the concentration dependence of these rheological properties. It was found that very similar values of C^* and C^{**} were obtained by using different measurement methods. The corresponding results may provide the basic information for its applications in enhanced oil recovery system.

2. Materials and methods

2.1. Materials

The dry sample of SM-A87 EPS was prepared with the method described in Ref. (Zhou et al., 2009). Fresh sample was stored in the refrigerator at 4 °C. The weight-average molecular weight was measured to be 3.76×10^6 g/mol with Gel Permeation Chromatographer (HP1100, Agilent) under the following conditions: column, 300 mm \times 7.8 mm I.D. TSK-GEL G4000PWXL (10 μ m particle size, 300 Å pore size, Tosoh Corporation, Minato-ku, Tokyo, Japan) with a TSK-guardcolumn; mobile phase, 0.1 M sodium chloride; flow rate, 0.5 mL/min; injection volume, 20 μ L of 0.1% SM-A87 EPS solution; temperature, 25 °C (Fig. S1 in supporting information).

2.2. Methods

2.2.1. Analysis of glycosyl composition and linkage

Glycosyl composition analysis: Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. An aliquot was taken from the sample and added to separate tubes with 20 μ g of Inositol as the internal standard. Methyl glycosides were then prepared from the dry sample following the mild acid treatment with 1 M HCl in methanol at 80 °C (16–18 h), followed by re-*N*-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The sample was then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80 °C (0.5 h). These procedures were carried out as previously described by Merkle and Poppe (1994) and York, Darvill, Mcneil, Stevenson, and Albersheim (1986). GC/MS analysis of the TMS methyl glycosides was performed on an AT 6890N GC interfaced to a 5975B MSD, using a Supelco EC-1 fused silica capillary column (30 m \times 0.25 mm ID).

Glycosyl linkage analysis: The sample was permethylated, depolymerized, reduced, and acetylated; and the resultant partially methylated alditol acetates (PMAAs) analyzed by gas chromatography-mass spectrometry (GC-MS). Initially, one aliquot of each sample was suspended in about 200 μ L of dimethyl sulfoxide. The sample was then permethylated by the method with treatment with sodium hydroxide and methyl iodide in dry DMSO. Following sample workup, the permethylated material was hydrolyzed using 2 M trifluoroacetic acid (2 h in sealed tube at

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