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Production and characterization of biopolymer schizophyllan using sago starch as a carbon source

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Abstract. A significant amount of oil gets left behind in the reservoir after the application of primary and secondary recovery methods. Water flooding is the most widely used secondary recovery method because of its availability and low cost. However, this method leads to high water/oil mobility ratio leaving behind most of the oil in the reservoir. To overcome this effect, polymers are added which increases the water viscosity due to their high molecular weight. Polymer flooding reduces the mobility ratio leading to a greater oil recovery. In recent years, biopolymers have attracted the attention of petroleum industries. There are very few reports on the production of biopolymers from fungi and even fewer among them have been produced commercially. Schizophyllan produced by the fungus *schizophyllum commune* in presence of a carbon source (usually glucose) via submerged fermentation process, has attracted attention of researchers recently. This biopolymer is currently available as an expensive grade material, whereby limiting its applications in the industry. We hereby report a method for schizophyllan production using cheaply available sago starch as a carbon source. Physico-chemical characterization of schizophyllan was carried out using Fourier Transform Infrared (FTIR) spectroscopy which showed characteristic spectral signature for the biopolymer. Using Thermo Gravimetric (TG) analysis, the biopolymer was observed to be thermally stable upto 125°C, showing potential applications in high temperature reservoir conditions. Gel Permeation Chromatography (GPC) revealed a high molecular weight of 14.73 million Dalton, while viscosity measurements show shear-thinning behaviour, desirable in polymer flooding applications. The obtained properties of the biopolymer, coupled with a cheap production process based on locally available carbon source, makes them ideal candidates for applications in polymer flooding for enhanced oil recovery.

1. Introduction

Water flooding is considered as the most widely used secondary recovery method because of its availability and low cost [1]. Although water flooding can help in improving the oil recovery, yet over the period of time it causes fingering effect because of the high water/oil mobility ratio [2-4]. Hence to improve the viscosity of water, polymers are added which can help in reducing the mobility ratio leading to a greater sweep efficiency. The success of polymer flooding depends on the concept of mobility ratio. The value of mobility ratio less than 1 implies a favourable mobility condition, where the polymer added to water increases the viscosity of water whereas the value of mobility ratio greater



than 1 implies unfavourable mobility conditions, where the viscosity of water is less than the oil and it bypasses the oil zone leading to the fingering effect [5-7].

In recent years biopolymers have attracted the attention of oil and gas companies. Biopolymers are complex chains of polysaccharides of microbial origin, synthesized by bacteria, fungi and yeasts. Literature studies have shown that, there exists few reports on production of biopolymers from fungi and one biopolymer among them which has been used commercially is schizophyllan. It is produced by the fungus named *schizophyllum commune*, a white rot ubiquitous mushroom which is widely spread all over Africa, America, Asia, Australia and Europe in presence of a carbon source by submerged fermentation process [8, 9].

Commercially produced schizophyllan utilizes glucose as a carbon source. Because of its tolerance to high temperature and salinity, good thermal and mechanical stability it has been gaining importance in recent years. But it is available as an expensive grade material [8, 10-16]. Since the cost of the chemicals plays a very important role in polymer flooding operations, researchers have been looking for alternative carbon sources for schizophyllan production. In the past many researchers have studied the utilization of carbon sources such as biomass, corn fibre, date syrup, beef extract, rice hull, corn steep liquor, etc [12, 15, 17-20] in schizophyllan production. Prior to applications under reservoir conditions, it is important to characterize their physico-chemical properties. To our knowledge, details on their physico-chemical properties especially at high temperature - high salinity conditions have not been reported. Further, there are no reports on applications of the schizophyllan produced from various sources in oil recovery applications. Also, none of them reports the utilization of sago starch, a cheap and widely available carbon source, for production of schizophyllan.

This article reports for the first-time production of schizophyllan using cheaply available sago starch as a carbon source and potato extract as a nitrogen source respectively. A detailed physico-chemical characterization of schizophyllan is carried out using FTIR, TGA, GPC and rheometry in order to determine chemical structure, thermal stability and fluid properties.

2. Materials and Method

2.1. Materials

The fungus *schizophyllum commune* was found growing on the fallen tree trunk at Universiti Teknologi Malaysia, Johor Bahru Campus. Sago starch is obtained from a local manufacturer in Malaysia.

The other materials utilized for our study are Potato Dextrose Agar (PDA) plates and potato extract.

2.2. Preparation

The fungus obtained was grown on PDA plates to get pure fungal culture medium, where a small part of the fungus was placed onto the middle of the PDA plate which is then incubated at $28 \pm 1^\circ\text{C}$ for a span of 7 days. These plates are stored in the refrigerator for all the future experiments.

The production media for growth of the fungi was composed of the following (g/l): 40 g of sago starch as the carbon source and 4 g of the potato extract as the nitrogen source. Dry heat sterilizing technique was used to sterilize the production media.

Small discs from the fungal culture medium were inoculated in 100 ml of the sterilized production media. These samples were then placed on an orbital shaker at room temperature for a period of 5 days. After the incubation period the samples were subjected to purification techniques mentioned in the next section of this article.

2.3. Production

The supernatants obtained after the incubation period were subjected to three different purification methods mentioned in the following sections.

2.3.1. Filtration. The supernatants obtained were filtered using the normal filtration technique under vacuum to separate out the solids.

2.3.2. Dialysis. The supernatants were subjected to dialysis using dialysis tubing having a molecular weight cut-off (MWCO) of 12 to 14 K Dalton. The supernatants were placed inside the tubing and suspended in 1000 ml deionized water for 48 hours with changing the water 3 to 4 times.

2.3.3. Freeze Drying. The supernatants were first filtered using normal filtration technique to separate out the solids and then combined with 95% ethanol in 1:1 volume ratio. This suspension was left over night for complete precipitation and the samples were then centrifuged at 4000 rpm for 40 minutes. The precipitates obtained were dissolved in deionized water and subjected to freeze drying at -50°C for 3 days in Heto FD4 Freeze Dryer.

2.4. Characterization studies

2.4.1. Viscosity studies. The samples obtained from the filtration, dialysis and freeze-drying techniques were subjected to viscosity measurements using RST Rheometer. 4.4 weight % of freeze dried sample was dissolved in 100 ml of deionized water before being subjected to viscosity measurements.

2.4.2. Structural characterization. The FTIR spectra of the produced biopolymer was recorded using IRTracer-100 Fourier Transform Infrared Spectrophotometer, Shimadzu in the range of $500\text{--}4000\text{ cm}^{-1}$.

2.4.3. Thermal stability. TGA was used to determine the thermal stability of the sample. This was carried out using PerkinElmer TGA 4000 Thermogravimetric Analyzer where the sample was subjected to heating at temperature ranges between $30\text{ to }950^{\circ}\text{C}$ under nitrogen flow at a constant heating rate of 10°C per minute. The weight loss was measured as a function of temperature.

2.4.4. Molecular weight determination. The molecular weight of the sample was determined using size exclusion chromatography technique by Agilent Technologies 1260 Infinity GPC. Here the samples were dissolved in an appropriate solvent which in this case was found to be Dimethyl Sulfoxide (DMSO) with slight heating at 60°C . These samples were then filtered and injected into a chromatography column. The separation of multi component mixture takes place in the column. The constant supply of DMSO was accomplished using a pump. The light scattering and viscometry detectors were used to determine the weight of a polymer in the eluting solvent.

3. Results and Discussion

3.1. Viscosity studies

Figure 1 shows the comparison of the viscosity profile for filtration, dialysis and freeze-dried samples using Brookfield RST-Rheometer. Each data point is an average of the three replicate measurements. The studies were performed at 30°C with shear rate varying between $0\text{ to }600\text{ s}^{-1}$. It can be observed from the graph that the viscosity profile of the samples obtained from the methods mentioned above are almost similar where all the samples purified using different techniques show non-Newtonian behaviour with the viscosity decreasing as a function of increase in shear rate. From this study it can be demonstrated that any of the three techniques mentioned above can be utilized for the purification of the biopolymer schizophyllan. For our simplicity we have followed the filtration technique. The shear thinning behaviour of schizophyllan makes it an ideal candidate for polymer flooding applications [14, 21].

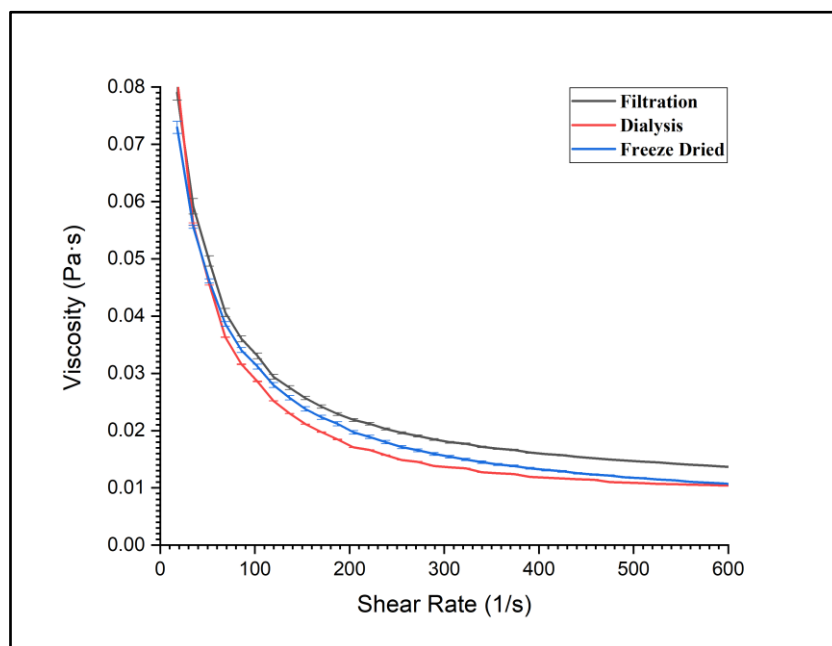


Figure 1. Comparison of the viscosity profile.

3.2. Structural characterization, thermal stability and molecular weight determination

The peaks for the FTIR spectrum obtained for the recovered schizophyllan as shown in figure 2 were found to be consistent with that of the structure for commercial schizophyllan data from the literature [12]. The recovered biopolymer has β -glycosidic bonds and pyranose rings which are the characteristic of schizophyllan [17, 22-24].

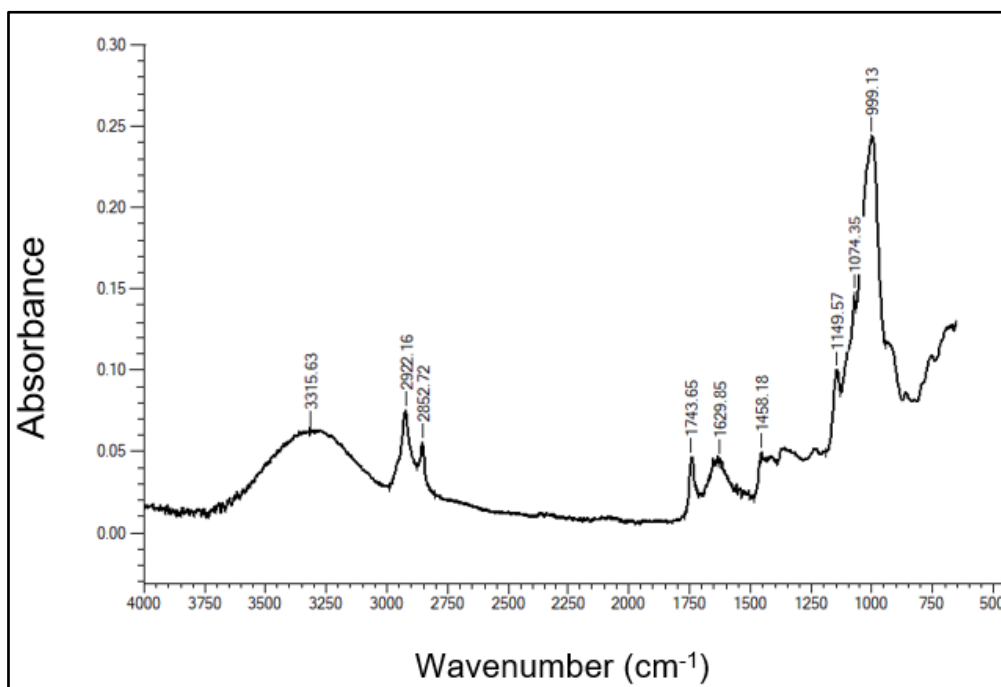


Figure 2. FTIR spectrum for the recovered schizophyllan.

The liquid sample after filtration was subjected to TG analysis. The graph of % weight loss as a function of temperature is shown in the figure 3. At 70°C, the sample showed slight degradation with a weight loss of around 6%. Further a weight loss of approximately 20% was observed when the temperature was increased to 100°C. The sample maintained good thermal stability up to a temperature of 125°C while retaining around 60% of its properties. Hence the recovered schizophyllan was found to be thermally stable up to a high temperature of 125°C.

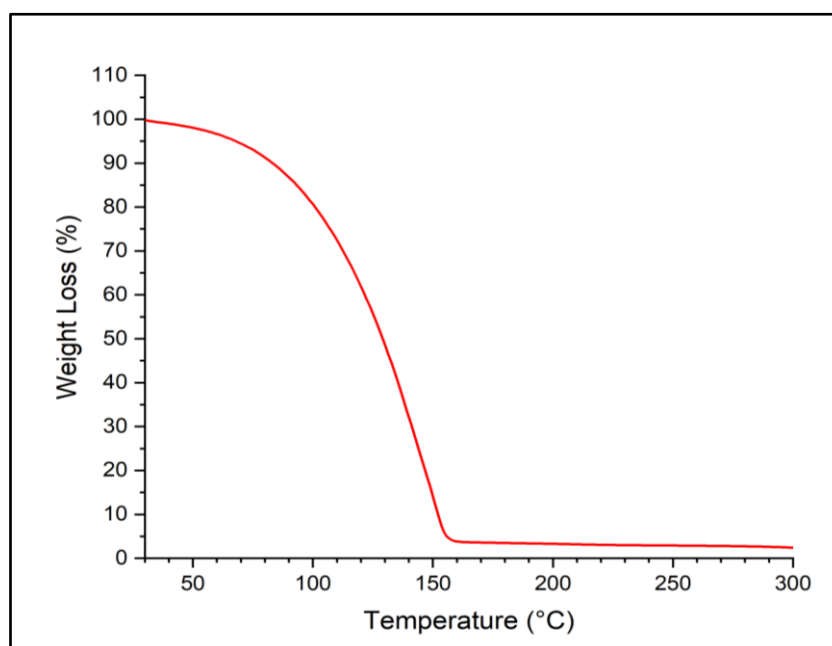


Figure 3. TG Analysis for the recovered schizophyllan.

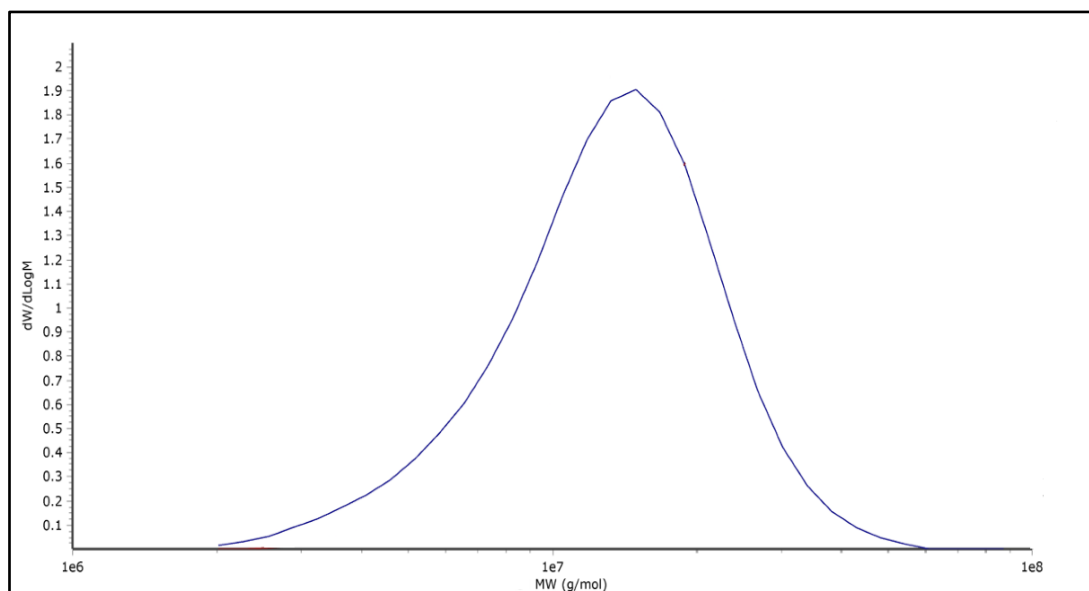


Figure 4. Molecular weight determination for the recovered schizophyllan.

The molecular weight of the recovered schizophyllan as obtained by GPC technique gave a peak value as shown in the figure 4. The weighted average molecular weight (Mw) for the sample was found to be 14.73 million Dalton and the value of polydispersity index of the sample to be 1.327

respectively. It can be observed that this value is consistent with the molecular weight range provided for commercial schizophyllan which is 6 to 12 million Dalton [9].

4. Conclusions

Our results show that cheaply available sago starch can be utilized as a carbon source in the schizophyllan production. The FTIR analysis of the recovered schizophyllan exhibited structural similarity in comparison to that of the commercial one. It also demonstrated good thermal properties up to a temperature of 125°C and hence can be applied in the high temperature reservoir conditions. The studies also showed that, the recovered schizophyllan has high molecular weight and hence exhibited good viscosity characteristics. Thus, sago starch can find its application as a carbon source in schizophyllan production, whereby making the production of the biopolymer economically sustainable and thereby, making schizophyllan an important candidate for polymer flooding applications.

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