Effect of Substrate Concentration Variation on Scleroglucan Production using Aerobic Fermentation

Nancy Siti Djenar and Bintang Iwhan Moehady
Chemical Engineering Department, Politeknik Negeri Bandung, Jln.Gegerkalong Hilir Ds. Ciwaruga,Bandung, Indonesia

Keywords: *S.rolfsii*, scleroglucan, liquid sugar, substrate

Abstract: *Sclerotium rolfsii* is known as one of the pathogenic fungi that causes some diseases in plants. In Indonesia the various studies that have been conducted on the fungus are still limited to prevention and control to reduce its pathogenic aspect. Scleroglucan is a biopolymer produced from *Sclerotium rolfsii* fermentation and has been used in several industries in various developed countries as both a thickener and emulsifier. In this study scleroglucan was produced from liquid sugar substrate using *S.rolfsii* InaCC F-05. The use of liquid sugar substrate with varying concentration can increase dry cell weight, yield of scleroglucan and conversion of 73.36%, 3.45% and 3.46% respectively. The acquisition of scleroglucan viscosity was 1.8500 to 2.5713 cP. While low, it can still be applied in chemical industries as a mixture for toothpaste and mouthrinse formulation.

1 INTRODUCTION

These days exopolysaccharides that were produced from varieties of microorganisms are widely used in some industries as emulsifers, gelling, and thickening agents. Scleroglucan one of the exopolysaccharides is produced from fermentation of *Sclerotium rolfsii* fungal (Survase, 2007). The properties and characteristics of scleroglucan are similar to xanthan gum. The difference is that in Indonesia scleroglucan is not yet well known, while xanthan gum has been widely used even though it still has to be imported.
Chemical structure of scleroglucan is depicted in Figure 1.

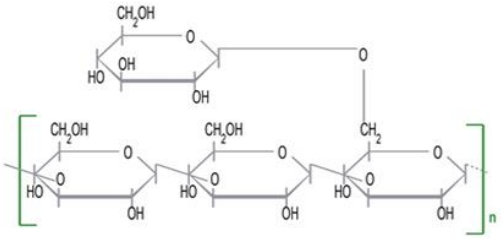

Figure 1: Chemical structure of scleroglucan (https://de.wikipedia.org/wiki/Scleroglucan).

Scleroglucan is composed of (1-3)- β-linked glucopyranosyl backbone with single (1-6)-β-linked glucopyranosyl branches on every third subunit.

In Indonesia *Sclerotium rolfsii* is better known as one of the pathogenic fungal that causes a number of diseases in plants such as peanuts, potatoes, tomatoes, soybeans, cabbage, onions, celery, corn and lettuce. This limits various studies that have been carried out regarding the fungal to prevention, control, and characterization in reducing the pathogenic properties of *Sclerotium rolfsii* (Yana, 2011 & Pudjihartati, 2007). The *S. rolfsii* metabolism process produces several enzymes including cellulases, phosphatidase, arabinase, exogalactanase, polygalacturanase, galactosidase and exomanase. The enzymes then convert raw materials into scleroglucan through this fermentation process (Castillo et.al, 2015).

In an industry that utilizes the fermentation process, the role of its medium is very important in increasing the microbial growth rate. PDB (Potato Dextrose Broth) is one of the medium commonly used for the growth of *S.rolfsii*. To support cell regeneration and productivity, the modified PDB is one of the best growth medium for *S.rolfsii* because it contains beef extract , K_2HPO_4 and KH_2PO_4, which function as a nitrogen source and buffer solution to maintain the pH of fermentation broth (Bhagat.I, 2011).

Djenar, N. and Moehady, B.
Effect of Substrate Concentration Variation on Scleroglucan Production using Aerobic Fermentation.
DOI: 10.5220/0009012504250429
In *Proceedings of the 7th Engineering International Conference on Education, Concept and Application on Green Technology (EIC 2018)*, pages 425-429
ISBN: 978-989-758-411-4

Temperature is a very important parameter that affect both culture growth and exopolysaccharide production. In this case the optimum temperature for scleroglucan production is in the range of 20-37°C and at 28^0C for culture growth. At less than 28°C, oxalic acid will gradually form which has an adverse effect on the production of scleroglucan (Survase, 2007).

pH affects the physiology of microorganism such as its solubility, nutrition and enzyme activity. The optimum pH for exopolysaccharide production can differ from the pH for culture growth. Generally the optimum pH for scleroglucan synthesis is between 4.0 and 5.5 (Castillo et al, 2015). Aeration and agitation optimization are the most important factors for controlling cell growth and scleroglucan production because it can increase the rate of formation of metabolites and oxygen from liquid medium to cells.

Substrates used in scleroglucan production are for example sucrose, condensed corn solubles, coconut water, molasses, sugar cane juice, and glucose (J.I Farina.1998, Fosmer.2010). Liquid sugar syrup produced from tapioca flour can be used as a substrate because it can shorten the scleroglucan production chain. Liquid sugar is easy to obtain even if its utilization is limited only as a raw material for the food and beverage industry, thus it is expected a diversification step will emerge from liquid sugar syrup which is made from local raw materials and spread throughout Indonesia (Djenar et al, 2017).

2 EXPERIMENTAL DETAILS

2.1 Microbial Preparation

At this stage regeneration of fungal *S.rolfsii* on :

a) Potato dextrose Agar (PDA) medium containing potato, dextrose and agar , incubated at 28°C for 5-7 days.
b) Modified PDA medium containing potato, dextrose, beef extract, KH_2PO_4, K_2HPO_4 and agar, incubated 28°C for 5-7 days.

2.2 Production of *S.rolfsii* Inoculum

2.2.1 Production of *S.rolfsii* inoculum in Potato Dextrose Broth (PDB)

Potato Dextrose Broth medium was prepared by dissolving 1.0 g potato, 0.1 g dextrose, 0.1 mg $CaCO_3$, 0.1 mg $MgSO_4$ $7H_2O$ in erlenmeyer containing 5 mL of distilled water. The medium then sterilized for 20 minutes at 121°C and 1.4 atm. The stock culture *S.rolfsii* in PDA was inoculated to the PDB medium and incubated at 28°C for 48 hours and stirred at 150 rpm. Furthermore, this active inoculum will be used to make *S. rolfsii* growth medium.

2.2.2 Production of *S.rolfsii* inoculum in modified Potato Dextrose Broth (PDB)

Potato Dextrose Broth medium was prepared by dissolving 1.0 g potato, 0.1 g dextrose, 0.1 mg $CaCO_3$, 0.1 mg $MgSO_4$ $7H_2O$, 0.0115 g K_2HPO_4, 0.0591 g KH_2PO_4 and 0.005 g beef extract in erlenmeyer containing 5 mL of distilled water. The medium then sterilized for 20 minutes at 121°C and 1.4 atm. The stock culture *S.rolfsii* in PDA was inoculated to the PDB medium and incubated at 28°C for 48 hours and stirred at 150 rpm. Furthermore, this active inoculum will be used to make *S. rolfsii* growth medium.

2.3 Experimental Work

In this step, aerobic fermentation was done in variation of growth medium at 170 rpm, pH 5, at 30-35 °C using 5% and 7% liquid sugar as substrate (Table 1)

Table 1: Variation of growth medium and substrate concentration on scleroglucan production.

No	Growth medium	Substrate concentration
1	PDB	5% Liquid sugar
2	PDB	7% Liquid sugar
3	Modified PDB	5% Liquid sugar
4	Modifiied PDB	7% Liquid sugar

Pasteurization. The pasteurization was done at 90°C for 25 minutes. The pasteurized fermentation was separated between scleroglucan solution and its cells using centrifugation.

Purification and precipitation. Precipitation of scleroglucan was done by adding an organic solvent of isopropyl alcohol (IPA) with a ratio of 3: 1 (v / v) to the supernatant. The obtained precipitate was then dried in an oven at 50°C, weighed, and the yield and % conversion were calculated.

Scleroglucan testing is done by measuring its viscosity. While the functional groups analysis uses FT-IR.

3 RESULT AND DISCUSSION

In this batch-based *S.rolfsii* fermentation the oxygen was produced from air which exists in the bioreactor by using a ratio of 1:5 between fermentation medium and the bioreactor (v/v) (Standbury P.F et al, 2000). At this stage curves were made to determine the production time of scleroglucan for this process, with pH between 4.0-5.5, temperature 30-35^{o}C with stirred at 170 rpm. The time needed to produce the maximum amount scleroglucan is shown in Table 2 below.

Table 2: The time needed to produce scleroglucan using liquid sugar substrate.

Growth medium	Substrate	The time needed to produce scleroglucan (hour)
PDB	5% liquid sugar	57
PDB	7% liquid sugar	68
Modified PDB	5% liquid sugar	76
Modified PDB	7% liquid sugar	65

In Table 3 It was shown that S.rolsii grown in PDB produced both low yield and conversion. The PDB only contains potatoes and dextrose which functions as carbon source. However, to increase the productivity, nitrogen source and others are required. In this PDB, an increase in substrate concentration causes a decrease both of yield and conversion. This happened as a result of the fermentation that was carried out in batch. This condition causes substrate repression to often occurs. In this experiment, 7% liquid sugar substrate repressed *S.rolfsii* metabolism, thus disrupting the enzyme synthesis (Egli, 2009).

A modified PDB is different in that changes in liquid sugar substrate with 5% to 7% concentration can increase yield and conversion about 3.45% and 3.46% respectively. Overall, when compared to PDB, there is an increase in yield and conversion of 17-18 g/L and 35-36% respectively. This is because the modified PDB contains beef extract as a source of nitrogen and K_2HPO_4 and KH_2PO_4. Nitrogen is a major component of amino acids, and these amino acids will form the proteins needed for cell metabolism – namely the growth and synthesis of enzymes, so as to increase the production of biopolymers (Survase,2007). Phosphorus is an important element for secondary cell metabolism. In fermentation medium, phosphorus is in the form of phosphate salts such as K_2HPO_4 or KH_2PO_4 which serve as a pH buffer. Potassium is the main inorganic cation in cells and is usually added as an inorganic salt. Potassium is a cofactor for several enzymes needed for carbohydrate metabolism. The results of scleroglucan production are as shown in the following Table 3.

As shown in Table 3 the value of scleroglucan viscosity was still low which was around 0.9585 - 2.5713 cP. However, according to Castillo, N. A., et al, (2015), scleroglucan with viscosity of 1.5-3 cP can still be applied in the chemical industries as a mixture for toothpaste and mouthrinse formulation.

The viscosity of an exopolysaccharide is affected by fermentation conditions such as temperature, pH, and oxygen content (Garcia-Ochoa, 2000). According to Castillo.A.N et al. (2015) and Survase S.A, et al. (2007), the rate of aeration used is usually at 2 vvm at a stirring speed of 180-600 rpm using air bubbles. In this study aeration was carried out by utilizing oxygen in the bioreactor with a ratio between fermentation medium with bioreactor of 1:5 (v/v) (Standbury,2000).

Table 3: Scleroglucan research result.

No	Growth Medium	Substrate Concentration	Fermentation time (hour)	Scleroglucan produced (g)	Yield (g/L)	Conversion (%)	Viscocity (cP)
1	PDB	5% liquid sugar	57	0.3785	1.893	3.785	0.9585
2	PDB	7% liquid sugar	68	0.2892	1.446	2.066	1.9358
3	Modified PDB	5% liquid sugar	76	3.5017	17.505	35.017	1.8500
4	Modified PDB	7% liquid sugar	65	3.6228	18.114	36.228	2.5713

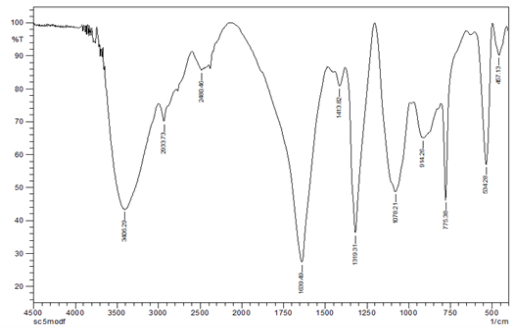

Figure 2: FTIR spectrum of scleroglucan from *S.rolfsii* fermentation using a modified PDB medium with a substrate of 5% liquid sugar.

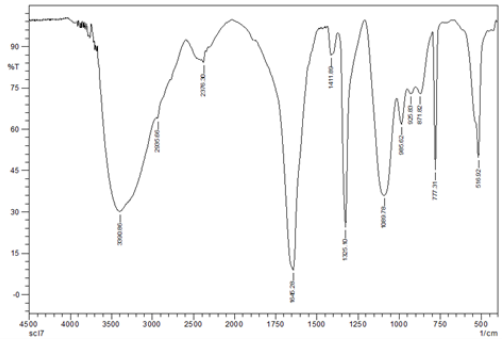

Figure 3. FTIR spectrum of scleroglucan from *S.rolfsii* fermentation using a modified PDB medium with a substrate of 7% liquid sugar.

Table 4: The comparison of functional groups between literature scleroglucan obtained using two types of substrates concentration.

No	Annotation	OH Stretching and Bending (cm^{-1})	CH Stretching and Bending (cm^{-1})	CH (cm^{-1})	CH Bending (cm^{-1})	GOC Glycosidic and CCOH Stretching (cm^{-1})
1	Farina, J.I. et. al, 2015	3400	2937	-	1475 – 1250	1000 – 1200
2	Casadei, M.A. et al, 2007	3422	-	1636	1420 – 1395	1079
3	Moehady, B. I et. al, 2016	3392	2933	-	1409 – 1382	1026 – 1153
4	Scleroglucan (using 5% liquid sugar)	3406	-	1639	1413 – 1319	1078
5	Scleroglucan (using7% liquid sugar)	3390	2935	1645	1411 –1325	1089

Based on the results of FTIR analysis, the presence of functional groups in the scleroglucan obtained from various fermentation process conditions can be recognized. Figure 2 and 3 showed the FTIR spectrum of scleroglucan using modified PDB with 5 and 7% liquid sugar substrate respectively.

The compatibility of functional groups between literature scleroglucan and scleroglucan of the study is shown in Table 4 below. Overall, this suggests that the fermentation of *S.rolfsii* using two types of the liquid sugar substrate concentration can produce scleroglucan.

4 CONCLUSION

The FTIR analysis, showed the compatibility of functional groups between literature scleroglucan and scleroglucan of the study. This suggests that the fermentation of *S.rolfsii* using two types of the liquid sugar substrate concentration will produce scleroglucan. The use both of 5% and 7% liquid sugar in modified PDB can increase yield and conversion about 3.45% and 3.46% respectively. The acquisition of scleroglucan viscosity was 0. 9585 to 2.5713 cP. While low, it can still be applied in chemical industries as a mixture for toothpaste and mouthrinse formulation.

.REFERENCES

Anisa, Yana, Nawangsih, & Abdjad Asih, 2011. *Pengaruh mulsa dan PGPR terhadap insidensi penyakit busuk pangkal batang (S.rolfsii) pada tanaman kedelai (glysine max (L) Merill)*. Institut Pertanian Bogor, Bogor

Bhagat, I., 2011. "Factors influencing mycelial growth of Sclerotium rolfsii", *Nepalese Journal of Biosciences*, Vol. 1, pp. 26-31.

Casadei, M.A., Matricardi P , Fabrizi G, Feeney M , & Paolicelli P, 2007. "Physical gels of a carboxymethyl derivative of scleroglucan: synthesis and characterization", *European Journal Of*

Pharmaceutics and Biopharmaceutics, Vol. 67, pp. 682-689.

Castillo, N. A., Valdez, A. L., & Fariña, J. I., 2015. "Microbial production of scleroglucan and downstream processing", *Frontiers In Microbiology*, Vol. 6, pp. 1106.

Djenar N.S and Wahyu. E., 2017. "The Effect of Substrate Modification on Xanthan Gum Production by Aerobic Fermentation Method". *Advanced Science Letters*, Vol. 23, No. 6, pp. 5678-5680

Egli, T., 2009. "Growth kinetics, bacterial". In M. Schaechter (Ed.), *Encyclopedia of Microbiology*, pp. 180-193.

Fariña, J.I., Siñeriz, F., Molina, O.E. & Perotti, N.I. 1998. "High scleroglucan production by Sclerotium rolfsii: Influence of medium composition". *Journal Of Biotechnology Letters*, Vol. 20, Issue 9, pp 825–831.

Fariña, J.I., Viñarta, S.C., Cattaneo, M., & Figueroa, L.I.C., 2008. "Structural stability of Sclerotium rolfsii ATCC 201126 β-glucan with fermentation time: a chemical, infrared spectroscopic and enzymatic approach". *Journal Of Applied Microbiology*, Vol.106, pp. 221-232.

Fosmer, A, Gibbons, W. R. & Heisel, N. 2010. "Reducing the Cost of Scleroglucan Production by Use of a Condensed Corn Solubles Medium," *Journal of Biotechnology Research*, Vol. 2, 2010, pp. 131-143.

García-Ochoa F, Santos VE, Casas JA, & Gómez E., 2000. "Xanthan gum: production, recovery, and properties". *Biotechnology Advances*, Vol. 18, pp. 549-579.

https://de.wikipedia.org/wiki/Scleroglucan.

Moehady, B. I., Djenar, N. S., Widyanti, E. M., 2016. "Produksi scleroglucan dari S.rolfsii menggunakan media gula cair", *Prosiding Seminar Nasional Teknik Industri*, Universitas Gajah Mada, pp M2-M9.

Peter F Stanbury, P. F., Whitaker, A, Hall, S., J. 2000. *Principles of Fermentation*. Butterworth-Heinemann, Oxford, Melboune.

Prasetyoningrum, Adnan, T, & Muin, A., 2012. "Potensi seduhan kompos untuk pengendalian penyakit layu S.rolfsii pada tanaman kedelai". Institut Pertanian Bogor, Bogor.

Pudjihartati, Sudarsono, E., Ilyas, Satriyas, & S, Siswanto, R., 2007. "Ketahanan kacang tanah dan tembakau terhadap infeksi S.rolfsii Sacc. dengan ekspresi enzim kitinase tinggi", Pusat Pelatihan Agrobisnis, Bioteknologi dan Sarana Industri, Bogor.

Survase, S. A., Saudagar, P. S., Bajaj, I. B., & Singhal, R. S. 2007. "Scleroglucan: Fermentative Production, Downstream Processing and Applications", *Food Technol.Biotechnol*. Vol. 45, No. 2, pp. 107-118.