

## Optimization of C/N ratio for production of heteropolysaccharide-7 by *Beijerinckia indica*

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### Abstract

Heteropolysaccharide-7 (PS-7) was produced by *Beijerinckia indica* HS-2001 under aerobic condition. Production of PS-7 was investigated under various ratios of glucose as carbon source to ammonium nitrate as nitrogen source. Maximal production of PS-7 was 7.13 g/l when concentrations of glucose and ammonium nitrate were 10 g/l and 0.3 g/l, respectively. But its conversion rate from glucose was as low as 7 %. The highest conversion rate of PS-7 was 46% when those of glucose and ammonium nitrate were 1.0 g/l and 0.3 g/l, respectively.

### Introduction

The molar repeat unit composition of PS-7 produced by aerobic submerged fermentation of *B. indica* was reported to be 72 % glucose, 18 % rhamnose, and 10 % galacturonic acid with an acetyl content of about 8.0~10.0 % (by weight). These properties indicate that PS-7 is suitable as a drilling fluid or additive for a thickened aqueous media for oil recovery. Other potential applications of PS-7 gum include dripless water-based latex, well-joint cement adhesives, paint compositions, as additives for foods and textile printing. In this work, the effect of optimization of C/N ratio for production of PS-7 was investigated.

### Materials and methods

**Bacterial strain** *Beijerinckia* formerly *Azotobacter indica* HP-2001 is UV-induced mutant of *B. indica* ATCC 21423. *B. indica* ATCC 21423 was obtained from the American Type Culture Collection (ATCC) and maintained on

slants of a mineral salts agar medium. The mineral salts medium (MSM) used for cell growth and the production of PS-7 contained the following components (g/l):  $K_2HPO_4$ , 5.0;  $MgSO_4 \cdot 7H_2O$ , 0.1;  $NH_4NO_3$ , 0.9g; Bacto peptone (Difco Lab., Detroit, MI), 0.5; and glucose, 20. The carbon source was autoclaved separately for 15 min at 120°C and added to the MSM under aseptic conditions.

**Production and purification of polymers** Starter cultures were prepared by transferring cells from agar slants to 100 ml of MSM with 2% (w/v) glucose in 500 ml Erlenmeyer flasks. These cultures were incubated at 30°C and for 24hr under aerobic condition and used to inoculate 50 ml of MSM with a carbon source in 250 ml Erlenmeyer flasks. Cultures were incubated for 40h under the same conditions used in preparing the starter cultures. Culture broths were centrifuged at 12,000 x g for 15 min at 4°C to remove cells. The supernatant was added to two volumes of 2-propanol, left to stand overnight at 4°C to precipitate the exopolymer, and centrifuged at 12,000 x g for 15 min to separate the precipitate. The precipitated material was repeatedly washed with isopropanol, acetone, and ether, dissolved in deionized water, and dialyzed against deionized water using dialysis tubing with a molecular weight cut off of 12,000 - 14,000 g/mol. After dialysis for 2-3 days with 4-5 times changes of deionized water, the solution was lyophilized.

**Analytical methods** To determine cell growth, culture broth were diluted appropriately with DW and were centrifuged at 12,000 x g for 15 min at 4°C. Pellets were washed with DW and were used to determine cell growth at 600 nm. To determine the biomass, cells were washed with distilled water and dry cell weight was measured by directly weighing the biomass after drying to constant weight at 100 - 105°C.

## Results and discussion

The effect of ammonium nitrate as the nitrogen source with 2% glucose as the carbon source on cell growth and production of PS-7 was shown in Table 1. Maximal production of PS-7 was 6.12 g/l when concentration of ammonium nitrate was 0.6 g/l. Its conversion rate from glucose to PS-7 was 30%.

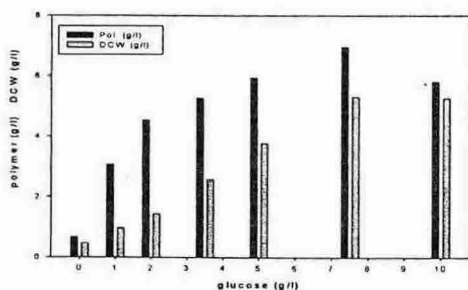
The effect of glucose as carbon source on production of PS-7 without ammonium nitrate was investigated (Fig. 1). Maximal production of PS-7 was 6.96 g/l when concentration of glucose was 7.5%. The highest conversion rate was 30% when concentration of glucose was 1.0%. Production of PS-7 with 0.3

g/l of ammonium nitrate and various concentration of glucose was shown in Fig. 2. Production of PS-7 increased with higher concentration of glucose whereas its conversion rate decreased. Maximal production of PS-7 was 7.13 g/l when concentration of glucose was 10% but its conversion rate was as low as 7%. The highest conversion rate of PS-7 was 46% when concentration of glucose was 1%. Production of PS-7 with 0.6 g/l of ammonium nitrate and various concentration of glucose showed similar result with 0.3 g/l of ammonium nitrate as shown in Fig. 3.

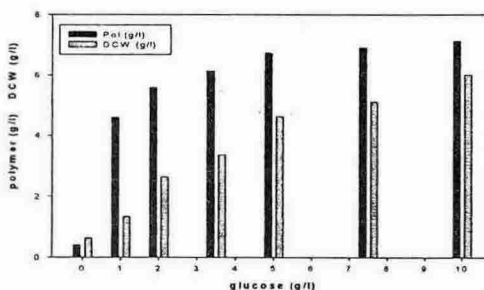
The highest conversion rate of PS-7 was 42% when concentration of glucose was 1%. Production of PS-7 with 0.9 g/l of ammonium nitrate and various concentration of glucose was show in Fig.4. Maximal production of PS-7 was 7.00 g/l when concentration of glucose was 10% but its conversion rate was as low as those with 0.3 g/l and 0.6 g/l of ammonium nitrate.

**Table 1.** Effect of various concentration of ammonium nitrate as the nitrogen source on cell growth and production of PS-7

NH <sub>4</sub> NO <sub>3</sub> (g/l)	Final status				
	pH	O.D(600nm)	DCW(g/l)	PS-7(g/l)	Yield(Y <sub>p</sub> /s)
0.0	6.51	3.92	1.45	5.42	0.27
0.3	6.32	5.83	1.46	5.83	0.29
0.6	6.11	4.85	1.47	6.12	0.30
0.9	4.80	4.06	1.30	2.89	0.15
1.2	4.89	4.04	1.28	2.17	0.11
1.5	4.78	4.02	1.28	1.92	0.10
1.8	4.74	3.95	1.30	1.58	0.08



**Fig. 1.** The effect of glucose as carbon source without ammonium nitrate in medium



**Fig. 2.** The effect of glucose with 0.3 g/l ammonium nitrate on cell growth and production of PS-7

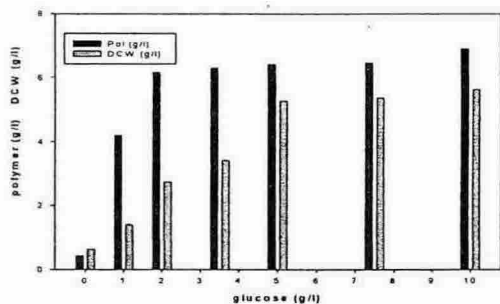


Fig. 3. The effect of glucose with 0.6 g/l ammonium nitrate on cell growth and production of PS-7

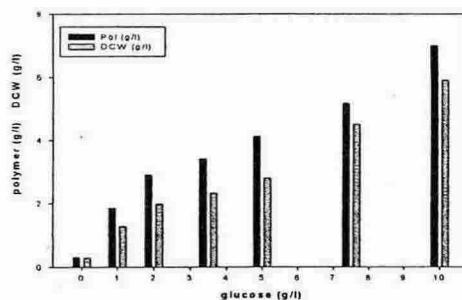


Fig. 4. The effect of glucose with 0.9 g/l ammonium nitrate on cell growth and production of PS-7

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