

Effect of the aeration rate and agitation speed on heteropolysaccharide-7 production by *Beijerinckia indica*

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Abstract

Effect of aeration rate and agitation speed on cell growth and the production of heteropolysaccharide-7 (PS-7) by *Beijerinckia indica* was investigated. Aeration rate and agitation speed in a 7L bioreactor ranged from 0.5 to 1.5 vvm and from 300 to 500 rpm, respectively. Higher agitation speed with an aeration rate of 0.5 vvm in the bioreactor resulted in maintenance of higher concentration of dissolved oxygen in the medium, which enhanced the production of PS-7. In this study with a 7L bioreactor, maximal production of PS-7 was 11.0 g/L and its conversion rate from 2% (w/v) glucose was 0.55 when the aeration rate and agitation speed were 1.0 vvm and 500 rpm, respectively. Proper aeration rate and agitation speed might enhance the production of PS-7 as well as reduce the time to reach maximal production.

Introduction

Heteropolysaccharide-7 (PS-7) is a water-soluble exopolymer produced by *Beijerinckia indica* var. *myxogenes*¹⁾ formerly *Azotobacter indica*. PS-7 was reported to consist of glucose and rhamnose by the gas chromatographic analysis²⁾ and to be degraded by the sphingonase that cleaves specific members of the gellan-related polysaccharides produced by some species of microorganisms^{3),4),5)}. The molar ratio of rhamnose to glucose in PS-7s with glucose-related sugars as the carbon source showed no significant variation of 1.0 to 4.5~4.7²⁾. PS-7 is a water-soluble exopolymer and generates about 10 times as higher viscous solution than xanthan.

The aim of this study was to investigate the effect of aeration and agitation on cell growth and the production of PS-7 in a bioreactor and established optimal

conditions involved in the dissolved oxygen in the medium.

Materials and methods

Bacterial strain and medium *Beijerinckia indica* HS-2001 is the UV-induced mutant of *B. indica* ATCC 21423, which was obtained from the American Type Culture Collection (ATCC), and maintained on slants of a mineral salts agar medium¹¹. The mineral salts medium (MSM) used for cell growth contained the following components (g/l): KH₂PO₄, 5.0; MgSO₄·7H₂O, 0.1; NH₄NO₃, 0.9g; Bacto tryptone (Difco Lab., Detroit, USA), 0.5; and glucose, 20. The carbon source was autoclaved separately at 120°C for 20 min and added to the MSM under aseptic conditions.

Production and purification of PS-7 Starter cultures were prepared by transferring cells from agar slants to 100 ml of the MSM with 2% (w/v) glucose in a 500 ml Erlenmeyer flasks. These cultures were incubated for 20 h at 30°C with a 200 rpm and used to inoculate 150 ml of the MSM with a carbon source (2%, w/v unless otherwise specified) in a 7L bioreactor. Culture broths were centrifuged at 12,000 x g for 30 min at 4°C to remove cells. The supernatant was added to two volumes of isopropanol and centrifuged at 9,000 x g for 1 h to separate the precipitate.

Analytical methods To determine cell growth and the production of PS-7, culture broth were centrifuged at 12,000 x g for 30 min at 4°C. Pellets were washed with DW and were used to determine cell growth at 650 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. Desalted samples after dialysis were used for quantitation of PS-7.

Results and discussion

Effect of the aeration rate and agitation speed on cell growth and the production of heteropolysaccharide 7 (PS-7) by *Beijerinckia indica* in a bioreactor was investigated. Aeration rate and agitation speed in a 7L bioreactor ranged from 0.5 to 1.5 vvm and from 300 to 500 rpm, respectively. The production of PS-7 with an aeration rate of 0.5, 1.0 and 1.5 vvm and different agitation speeds was shown in Fig. 1, 2 and 3 respectively. Generally speaking, higher agitation speed might result in higher concentration of dissolved oxygen in the medium, which

promoted cell growth and enhanced to produce PS-7 by *B. indica* HS-2001. Maximal production of PS-7 with an aeration rate of 0.5 vvm was 9.0 g/L when agitation speed was 500 rpm shown in Fig. 1. Its conversion rate from 2% (w/v) glucose was 0.45 (Table 1).

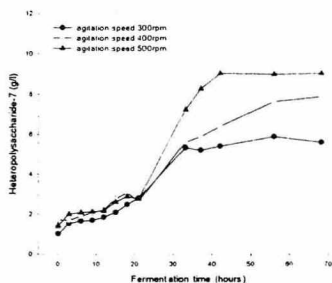


Fig. 1. The effect of aeration rate of 0.5vvm with agitation speed on the production of PS 7 by *B. indica* HS2001

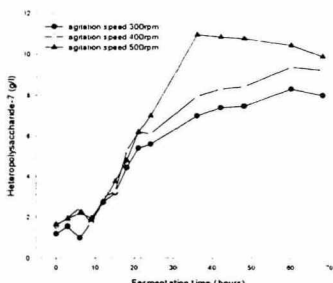


Fig. 2. The effect of aeration rate of 1.0vvm with agitation speed on the production of PS 7 by *B. indica* HS2001

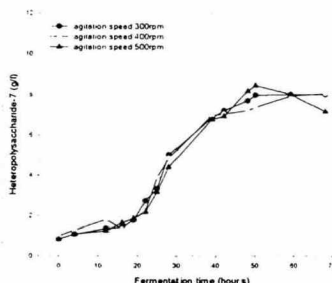


Fig. 3. The effect of aeration rate of 1.5vvm with agitation speed on the production of PS 7 by *B. indica* HS2001

The time to reach the maximal production of PS-7 also reduced with higher agitation speed. Maximal productions of PS-7 with an aeration rate of 1.0 and 1.5 vvm were 11.0 and 8.4 g/L when both agitation speeds were 500 rpm, respectively, shown in Fig. 2 and 3. Their conversion rates were 0.55 and 0.42, respectively (Table 1). In this study with a 7L bioreactor, optimal condition of aeration rate and agitation speed involved in the dissolved oxygen in the medium, which affect cell growth and the production of PS-7, were established.

Table. 1. Final results for the production of heteropolysaccharide-7 by *B.indica*^{a)}

Aeration(vvm)	0.5			1.0			1.5		
Agitation(rpm)	300	400	500	300	400	500	300	400	500
Final pH	5.64	5.87	6.33	5.65	5.92	6.40	5.71	5.91	5.91
Process time(hr)	68	68	68	68	68	68	68	68	68
Process time for maximal production(hr)	56	68	42	60	60	36	68	68	50
Maximal production(g/l)	5.88	7.88	9.04	8.32	9.40	10.96	8.00	8.04	8.44
Conversion rate	0.29	0.39	0.45	0.42	0.47	0.55	0.40	0.40	0.42
Productivity(g/l · h ⁻¹)	0.10	0.16	0.22	0.14	0.16	0.30	0.12	0.12	0.17

a) Used the 2%(w/v) glucose as carbon source and 0.1%(w/v) soybean pomace as nitrogen source

Maximal production of PS-7 with a 7L bioreactor was 11.0 g/L when an aeration rate and agitation speed were 1.0 vvm and 500 rpm, respectively. The time to reach maximal production of PS-7 under above conditions was 36 h and productivity of PS-7 by *B. indica* HS-2001 with a 7L bioreactor was 0.30 g/L · h.

References

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