Production of *Beijerinckia indica* HS-2001 in Fed-batch and continuous culture.

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Abstract

As a result of broth substitutions when each culture-mediums were difference, whole culture-medium was found to be best feeding solution for production of PS-7 by *B. indica*. Maximal production of PS-7 was 10.0 g/ℓ and its conversion rate from 2% (w/v) glucose to PS-7 was 50%. After 48 hr, 50%(v/v) medium of working volume began to substitute in 7L jar fermenter. Production of PS-7 increased after 48hr, recovered productivity of PS-7. Following this preliminary culture, the resultant culture was subjected to continuous flow conditions controlled that the dilution rate were 0.01 ~ 0.04 h^{-1}. Production of PS-7 increased at dilution rate 0.0100 h^{-1} whereas productivity of PS-7 decreased gradually in dilution rate 0.0200 ~ 0.0400 h^{-1}. Maximal production of PS-7 was 10.0 g/ℓ in continuous culture.

Introduction

Heteropolysaccharide-7(PS-7) is a water-soluble exopolymer produced by *Beijerinckia indica* var. myxogenes[2], formerly *Azotobacter indica*. PS-7 was reported to consist of glucose and rhamnose by the gas chromatographic analysis[1] and to be degraded by the sphinganase that cleaves specific members of gellan-related polysaccharides produced by some species of microorganisms. The molar ratio of rhanmose to glucose in PS-7s with glucose-related sugars as the carbon source showed no sigificant variation of 1.0 to 4.5 ~ 4.7[2]. PS-7 is a water-soluble exopolymer and generates high viscosity solutions(about twice that of xanthan). The viscosity of PS-7 solutions is stable over the temperature range from 4℃ ~ 93℃ and a pH range from 3.0 to 12.0. PS-7 has a good pseudoplasticity, which the viscosity of PS-7 decreases as the shear rate increases. PS-7 is incompatible with cationic or polyvalent ions at high pH which results in gel formation. These properties

indicate that PS-7 is suitable as a drilling fluid or an additive for a thickened aqueous media for oil recovery.[2] Other potential applications of PS-7 include dropless water-base latex, well-joint cement adhesives, and textile printing.

Materials and methods

Bacterial strain *Beijerinckia* (formerly *Azotobacter*) *indica* HS-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[2]. The mineral salts medium (MSM) used for cell growth and 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, 0.9; Bacto peptone (Difco Lab., Detroit. MI), 0.5; and glucose, 20.

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 24 hr under aerobic condition and used to inoculate 50 ml of MSM with a carbon source in 250 ml Erlenmeyer flasks. Cultures were incubated for 40hr under the same conditions used in preparing the starter cultures. Starter cultures was used for an inoculum for 5 ℓ of medium in 7 ℓ fermenter. The carbon source was autoclaved separately for 30 min at 120℃ and added to the MSM under aseptic conditions. Inoculum size was 5% (v/v). Culture were incubated for 4 days at 30℃, aeration rate of 1 vvm and agitation rate of 200~500 rpm. Culture broths were centrifuged at 12,000 × g for 15 min at 4°C to remove cells. The supernatant was added to two volumes of isopropanol, left to stand overnight at 4°C to precipitate the exopolymer, and centrifuged at 12,000 × 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.

Culture condition of continuous culture Experiments employed a 7L bioreactor with two six-bladed impellers and 3 baffles. The dilution rate ranged from 0.1 to 0.4 h^{-1} working volume was 4L. Aeration rate and agitation speed were 1vvm and 500rpm.

Results and Discussion

The effect of broth substitution on cell growth and production of PS-7 by *B. indica* was

examined (Fig. 1). Substitutions of medium used in this study were 1) 2%(w/v) glucose 2) 2%(w/v) glucose, 0.6 g/ℓ NH_4NO_3 and 1.0 g/ℓ soybean pomace 3) 2%(w/v) glucose, 0.6 g/ℓ NH_4NO_3, 0.1 g/ℓ $MgSO_4 \cdot 7H_2O$ and 1.0 g/ℓ soybean pomace. The pH was adjusted to 6.80 before sterilization. Maximal production of PS-7 was 10.0 g/ℓ and its conversion rate from 2% (w/v) glucose to PS-7 was 50%. After 48 hr, 50%(v/v) medium of working volume began to substitute in 7L jar fermmeter. Production of PS-7 increased after 48 hr, recovered productivity of PS-7.

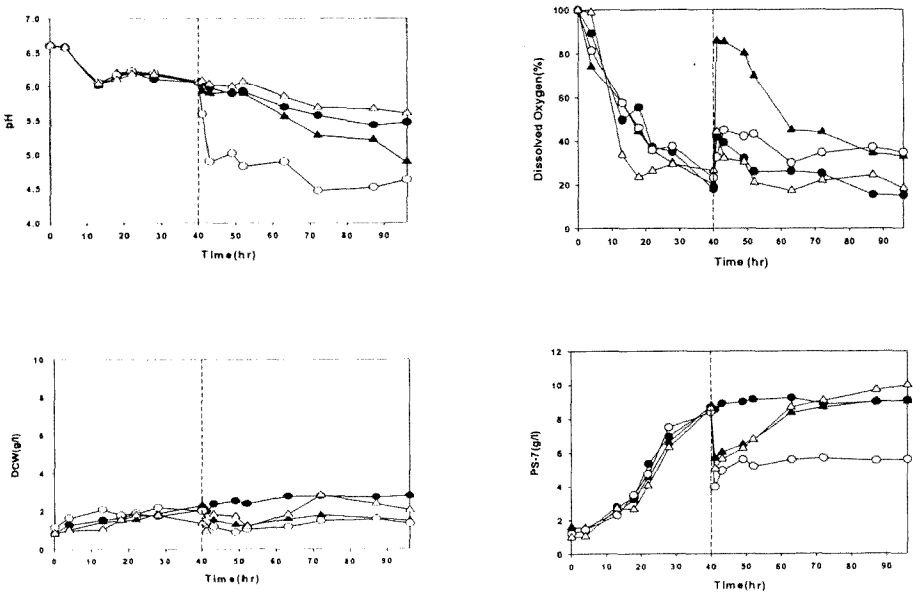

Fig. 1. Effect of broth substitution on production of PS-7 by *B. indica*
(● no substitution; ▲ 2% Glucose pH 6.8; ○ 2% Glucose+NH_4NO_3 0.6 g/ℓ +soybean pomace 1.0 g/ℓ pH 6.8; △all culture medium pH 6.8)

Following this preliminary culture, the resultant culture was subjected to continuous flow conditions controlled that the dilution rate were 0.01 ~ 0.04 h^{-1}. The PS-7 was produced by *B. indica* with continuous culture(Fig. 2). The dilution rate were 0.01, 0.0125, 002 and 0.04. Production of PS-7 and dry cell weight were increased for continuous culture at dilution rate of 0.01 h^{-1}. In continuous culture at dilution rate of 0.0125 h^{-1} resulted in maintenance of higher production of PS-7 and dry cell weight. It occurred that wash out of cells at dilution rates higher than 0.02 h^{-1} and production of PS-7 was decreased. At

dilution rate of 0.0125 h^{-1}, it was obtained the maximal mount of exopolymer. Optimal dilution rate of production of PS-7 was 0.0125 h^{-1} in continuous culture.

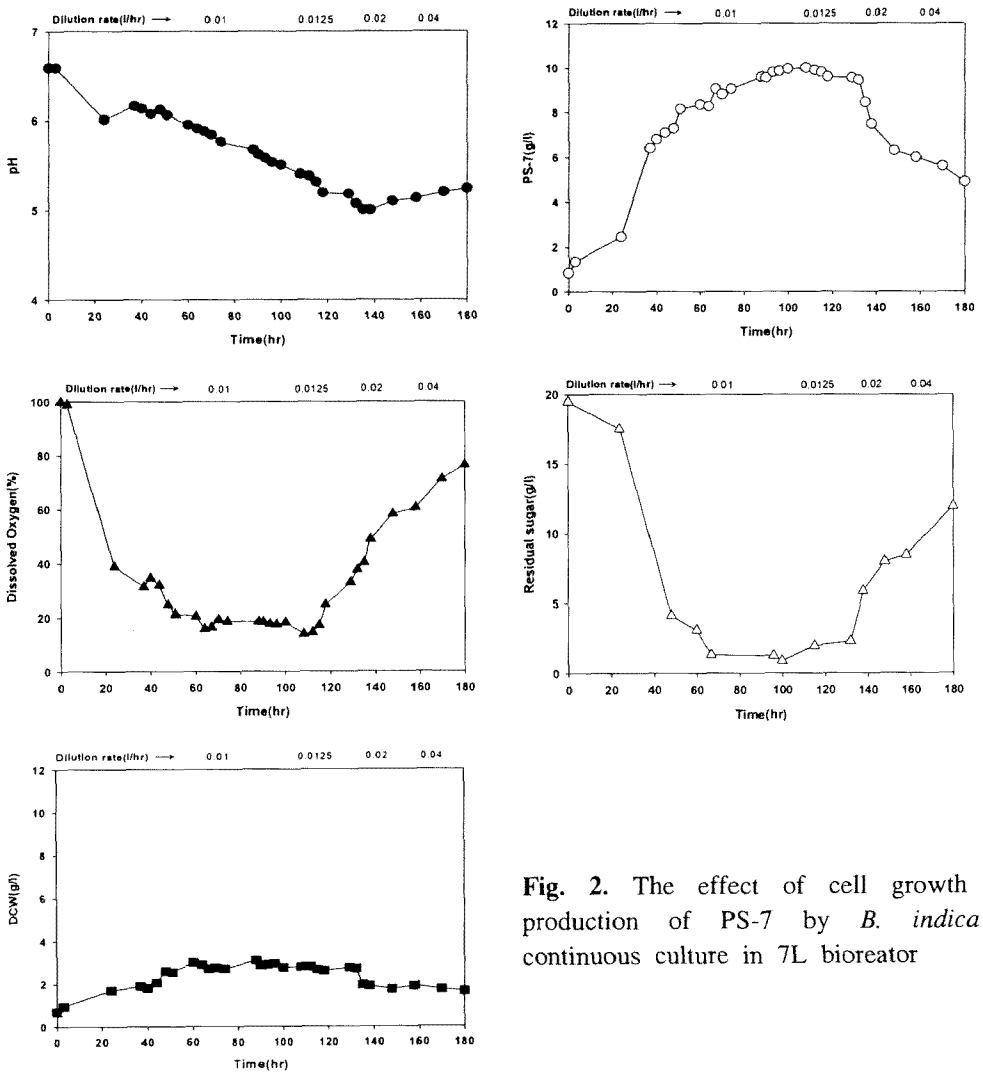

Fig. 2. The effect of cell growth and production of PS-7 by *B. indica* in continuous culture in 7L bioreator

References

1. Lee, J.W., Yeoman, W.G., Allen, A.L., Kaplan, D.L., Deng, F., Gross, R.A. Compositional consistency of a heteropolysaccharide-7 produced by *Beijerickia indica*. Biotechnol lett 1997;19(0):803-7.
2. Kang, K.S., McNeely W.H. "Polysaccharide and bacterial fermentation process for its preparation." (1976) U S patent, 3, 960, 832.