Production of heteropolysaccharide-7 by *Beijerinckia indica* HS-2001 with continuous culture

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Abstract

Maximal production of heteropolysaccharide-7(PS-7) with a batch culture for 48 hr was 10.0 g/ℓ and its conversion rate from 2% (w/v) glucose to PS-7 was 50%. After substitution of media, production of PS-7 continued and reached its maximal production. The highest productivity occurred when the fresh medium, which contained all ingredients, for the production of PS-7 was substituted. Higher production of PS-7 was maintained at a dilution rate of 0.0125, which was established as the optimal dilution rate for the production of PS-7 by *B. indica* HS-2001.

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 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 significant 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[3]. 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*, which was obtained from the American Type Culture Collection (ATCC). 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 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 40 hr 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 2~4 days at 30℃ with 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 conditions for continuous culture Experiments employed a 7 ℓ bioreactor with two six-bladed impellers and 3 baffles. The dilution rate ranged from 0.01 to 0.04 h^{-1} working volume was 4 ℓ. Aeration rate and agitation speed were 1vvm and 500rpm.

Results and Discussion

The effect of medium substitution after 48 hr on cell growth and production of PS-7 by *B. indica* was examined (Fig. 1). Maximal production of PS-7 with a batch culture for 48 hr 10.0 g/ℓ and its conversion rate from 2% (w/v) glucose to PS-7 was 50%. Substituted solutions 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 and 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, which is the medium for the production of PS-7. The volume of substituted solution was a half of original working volume. The pHs of substituted solutions were adjusted to 6.80 before sterilization. After substitution of medium, production of PS-7 continued and reached its maximal production. The highest productivity occurred when the substituted solution contained all ingredients for the production of PS-7.

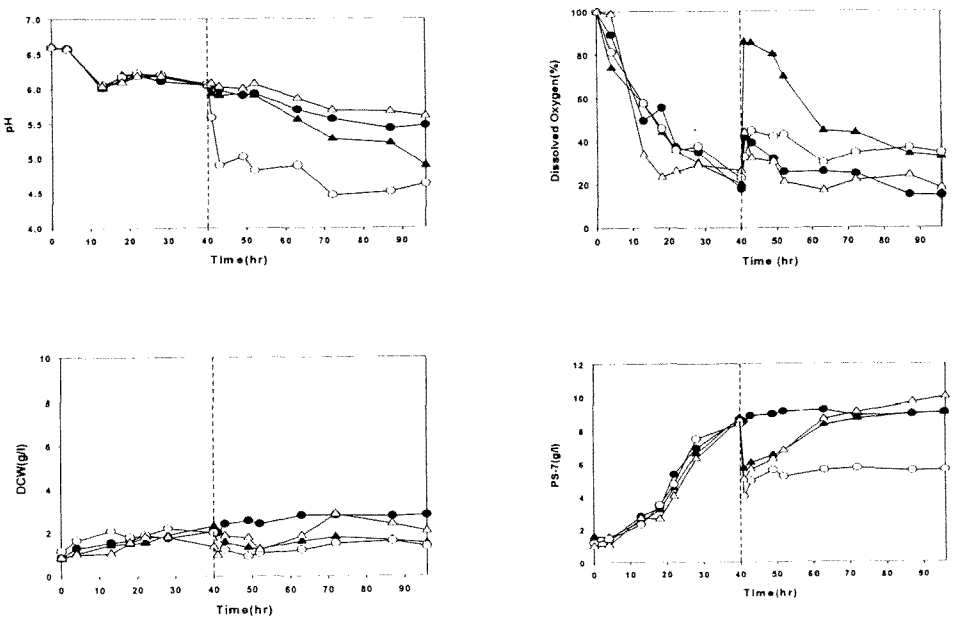

Fig. 1. Effect of medium substitution on production of PS-7 by *B. indica* (●, no substitution; ▲, 2% Glucose; ○, 2% Glucose, 0.6 g/ℓ NH_4NO_3 and 1.0 g/ℓ soybean pomace and △, new culture medium)

Continuous culture of *B. indica* HS-2001 for the production of PS-7 was performed in a 7 ℓ bioreactor (Fig. 2). Feeding material was the fresh medium for the production of PS-7. The dilution rate were 0.0100, 0.0125, 00200 and 0.0400. Production of PS-7 and dry cell weight gradually increased with a dilution rate of 0.01 h^{-1}. Higher production of PS-7 was maintained at a dilution rate of 0.0125. Above a dilution rate of 0.02 wash out of cells occurred and production of PS-7 decreased. Optimal dilution rate for the production of PS-7 was 0.0125 h^{-1} in the continuous culture of *B. indica* HS-2001.

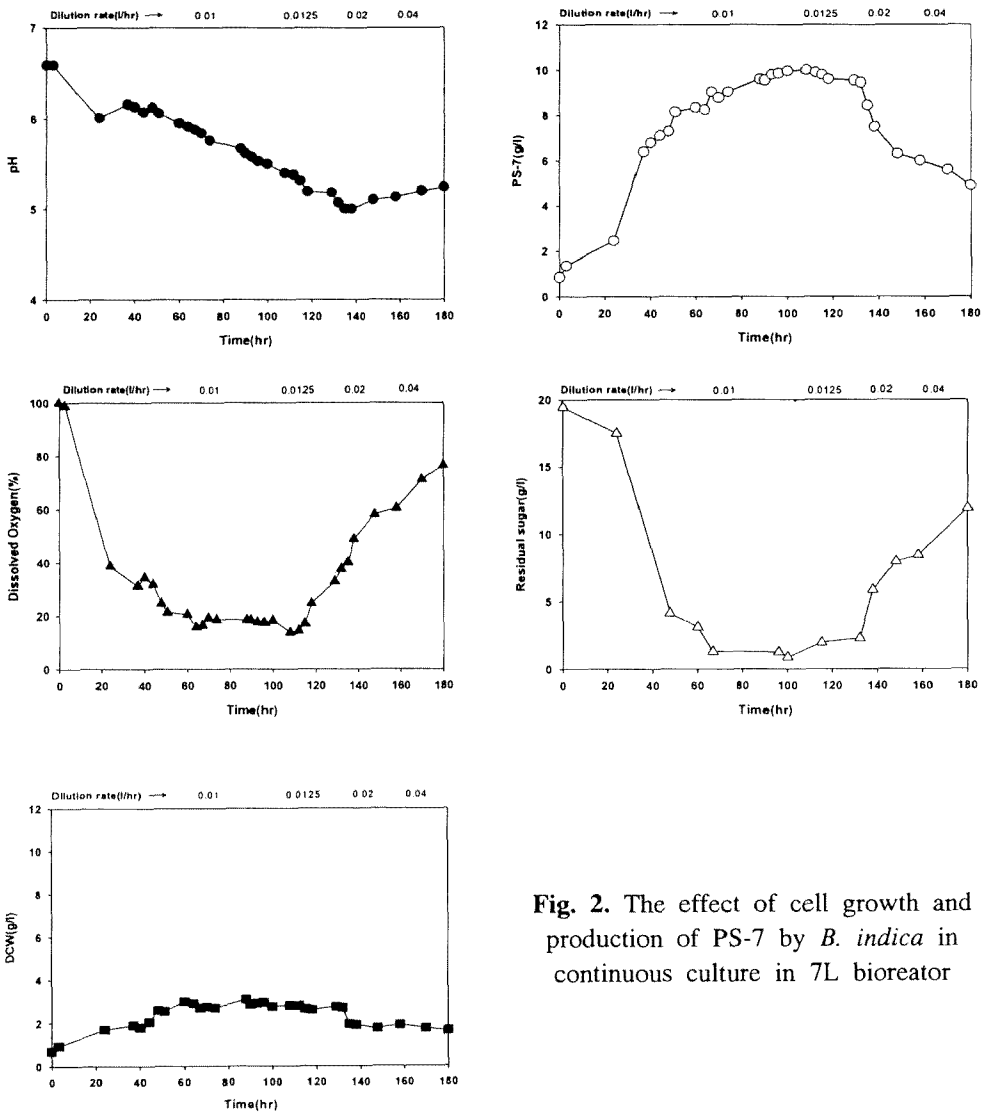

Fig. 2. The effect of cell growth and production of PS-7 by *B. indica* in continuous culture in 7L bioreator

References

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