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Effect of agitation speed on production of exopolymer by *Pseudomonas elodea* NK-2000

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Abstract

Extracellular polysaccharide was produced by *Pseudomonas elodea* NK-2000 under nitrogen limitation and aerobic condition. The effect of agiatation speed on cell growth and production of exopolymer was investigated. The agitation speed of 7.5 L fermentor ranged from 200 to 500 rpm. Production of exopolymer increased with higher agitation speed. Maximal cell growth and production of exopolymer from 2% glucose were 3.35 g/l and 3.80 g/l, respectively when agitation speed was 400 rpm.

Introduction

The extracellular polysaccharide, commercially known as PS-60 or gellan gum, was produced by *Pseudomonas elodea* . The gellan gum consists of linear repeating tetrasaccharide [→3)-β-D-Glc-(1→4)-β-D-GluA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→] composed of D-glucose (Glc), D-glucuronic acid (GlcA), and L-rhamnose residues (Rha)[6]. Gellan gum contains *O*-acetyl group that are removed by heating at pH 10. Gellan gum with low acetyl groups products a bittle, firm, and optically clear gel. According to its property to produce a thermoreversible gel, gellan gum can be substituted for agar. Due to diversity of its structure and properties, gellan gum has a wide range of applications in the food, pharmaceutical and other industries as texturizing, stabilizing, thickening, emulsifying and gelling agents. Also, gellan gum has been used for enzyme and cell immobilization and gel electrophoresis.

Materials and Methods

Bacterial strain.

Pseudomonas elodea NK-2000 is a UV-induced mutatnt of *P. elodea* ATCC

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Medium and culture condition.

The medium used for cell growth and exopolymer production contained the following composition (g/l): Glucose, 20; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; NH_4NO_3, 0.9; Bacto peptone (Difco Lab., Chicago, USA), 0.5; and mineral salt solution, 0.1ml. The mineral salt solution contained the following composition (mg/l): $MnCl_2 \cdot 4H_2O$, 1.8g; $FeSO_4 \cdot 7H_2O$, 2.487g; H_3BO_3, 0.285g; $CuCl_2$, 27; $ZnCl_2$, 21; $CoCl_2 \cdot 6H_2O$, 74; $MgMoO_4$, 23 and sodium tartrate (dihydrate), 2.1g. The pH of medium was adjusted to 6.5 - 6.8 before sterilization. The carbon source was autoclaved separately for 15 min at 121℃ and added to the medium under aseptic conditions.

Production of exopolymer.

Starter cultures were prepared by transferring cells agar slants to 100 ml of the medium with 2% (w/v) glucose in 500 ml Erlenmeyer flasks. These culture were incubated for 1 days at 30℃ and 200 rpm. This starter culture was used as inoculum for 5 L medium with 2% glucose (w/v) in a 7.5 L fermentor. Culture was incubated at 30℃ for 3 days under nitrogen limitation. Inoculumn size was 5% (v/v) and agitation speed ranged from 500 to 200 rpm with aeraton rate of 1 vvm. Samples were periodically withdrawn from the culture to determine cell growth and production of exopolymer.

Recovery of PS-60.

Deacetylated PS-60 was prepared by heating culture broth at 95℃ for 10 min. The pH was adjusted to 10 by 1N NaOH and then neutralized with 1N HCl. The pretreated broth was centrifuged at 10000 x g for 10 min to separate the cells[5]. The supernatant was mixed with 2 vol. of isopropanol. This precipitated material was repeatedly washed with acetone and ether and dried.

Analytical methods. Cell growth and production of exopolymer were determined by directly weighing biomass and exopolymer after drying to constant weight at 100- 105℃. The optical density (OD) was measured by spectrophotometer at 600nm.

Result

Cell growth and production of exopolymer in a 7.5 L fermentor with various agitation speeds was shown in Fig. 1, 2, 3, and 4.

Cell growth and production of exopolymer varied with agitation speed.

The final pHs of culture broth with different agitation were under 4. At the agitation speed of 200 and 300 rpm, dissolved oxygen decreased slowly.

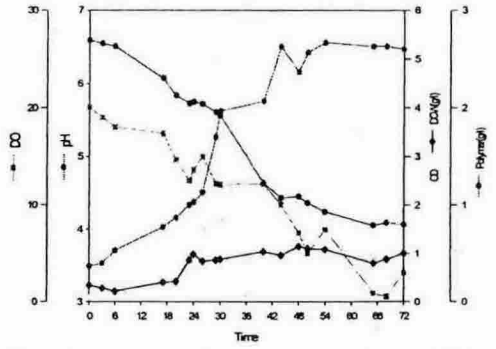

Figure 1. Cell growth and production of exopolymer at 200 rpm

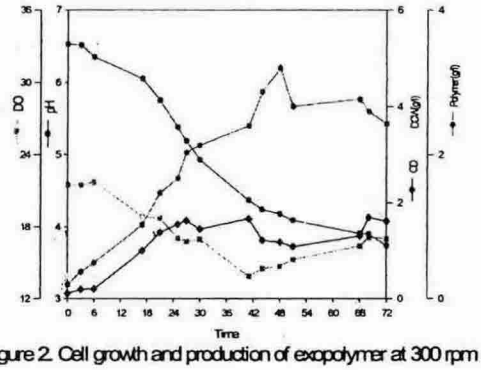

Figure 2. Cell growth and production of exopolymer at 300 rpm

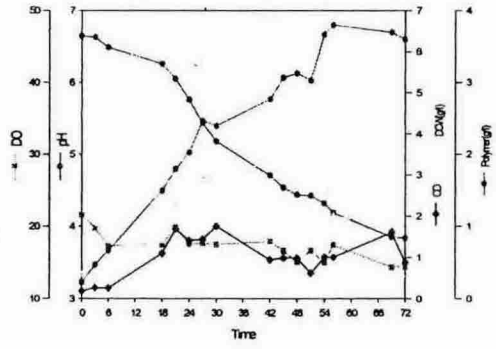

Figure 3. Cell growth and production of exopolymer at 400 rpm

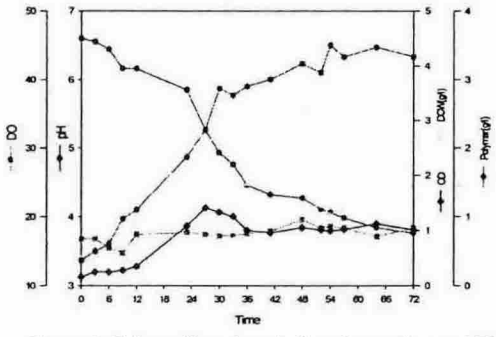

Figure 4. Cell growth and production of exopolymer at 500 rpm

Cell growth and production of exopolymer with various agitation speed were compared in Fig. 5 and 6.Production of exopolymer increased with higher agitation speed. Maximal cell growth and production of exopolymer from 2% glucose were 3.35 g/l and 3.80 g/l, respectively when agitation speed was 400 rpm.

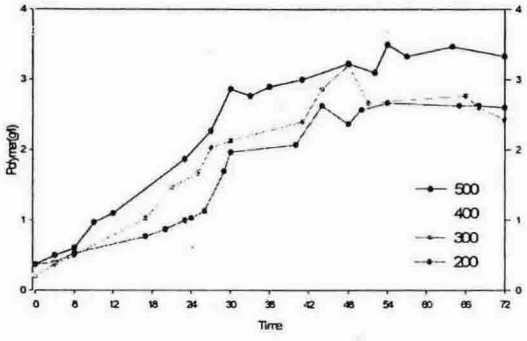

Figure 5. Effect of agitation speed on production of exopolymer

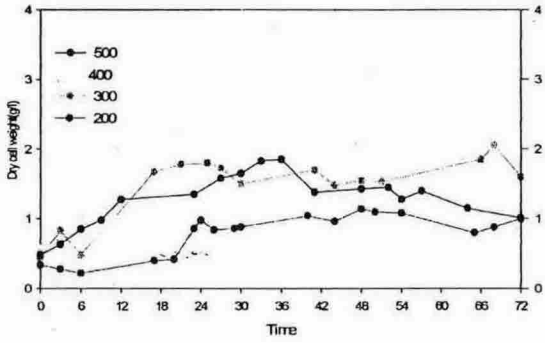

Figure 6. Effect of agitation speed on cell growth

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