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Optimization of β-carotene production by *Rhodotorula glutinis* DM28 in fermented radish brine

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Abstract

A face-centered central composite design was applied to optimize a cultivation condition for improved β-carotene production by *Rhodotorula glutinis* DM28 in a stirred tank reactor using 30 g/l total soluble solid of fermented radish brine as a sole substrate. The experiments were performed with regression models, where temperature, pH and dissolved oxygen were considered as variables. Results showed that an optimum condition for β-carotene production of the yeast was at 30 °C, pH 6 and 80% dissolved oxygen. Under this condition, the yeast yielded 2.7 g/l biomass and the maximum β-carotene of 201 μg/l after 24-h fermentation indicating approximately 15% higher than those under an initial condition (2.3 g/l and 178 μg/l, respectively).

Keywords: β-Carotene; *Rhodotorula glutinis*; Statistical experimental design; Fermented radish brine; Optimization

1. Introduction

β-Carotene is an orange-yellow pigment of carotenoid family recently demonstrating anticancer and antioxidant properties (Astorg, 1997). It is commercially used as an additive in food, feed, cosmetic and pharmaceutical products (Matelli et al., 1990). It is commonly found in plant and animal tissues and produced by a wide range of microorganisms such as *Sphingomonas* sp. (bacteria), *Dunaliella bardawil* (algae), *Blakeslea trispora* (fungi) and *Rhodotorula* spp. (yeast) (Britton, 1983; Goodwin, 1992; Silva et al., 2004).

Rhodotorula glutinis is widely known as a β-carotene-producing yeast (Simpson et al., 1975). It is potentially useful for industries since it is able to grow in various cheap agricultural raw materials such as sugar cane juice, peat extract, whey, grape must, beet molasses, hydrolyzed mung bean waste flour, soybean and corn flour extracts and sugar cane molasses for carotenoid production (Aksu and Eren, 2005; Bhosale and Gadre, 2001a; Buzzini and Martini, 1999; Frengova et al., 1994; Matelli et al., 1990; Martin et al., 1993; Park et al., 2005; Tinoi et al., 2005). It has an advantage over algae, fungi and bacteria due to its unicellular and relatively high growth rate with utilizing low-cost fermentation media.

Fermented radish is one of fermented vegetables popularly consumed in the Oriental countries. Tons of fermented radish have been annually produced in Thailand for self consumption and exports. Its production generates a considerable amount of brine as a waste which requires treatments before its discharge. Several reports have showed that the brine generated from the fermented vegetable industry could be a substrate for the growth and β-carotene production of *Rhodotorula* sp. (Shih and Hang, 1996; Suntornsuk, 2000; Vajang and Suntornsuk, 2001). However, this brine has not been investigated for β-carotene production by *Rhodotorula* sp.

Statistical experimental design is a powerful statistical technique generally employed for medium or process optimization of microbial fermentation. It had successfully applied for optimization of carotenoid production from *Rhodotorula* sp. (Bhosale and Gadre, 2001b; Buzzini,

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2000; Park et al., 2005; Vijayalakshmi et al., 2001). This method was, therefore, used in the current study to determine an optimum cultivation condition of β-carotene production by *R. glutinis* DM28 using fermented radish brine as a medium.

2. Methods

2.1. Microorganism and media

R. glutinis DM28 from the collection of the Department of Microbiology, KMUTT, was used. It was isolated from fermented vegetable brine and was found to produce β-carotene (Maneewatthana et al., 2000; Vajang and Suntornsuk, 2001). The yeast was maintained on a yeast and malt extract agar slant (3 g/l yeast extract, 3 g/l malt extract, 5 g/l peptone, 10 g/l glucose and 120 g/l agar) at 4 °C until used.

Fermented radish brine was supplied by a local pickle factory and kept at 4 °C until used. Total soluble solid (TSS), NaCl content, total acidity, nitrogen content, total sugars, phosphorus content, biochemical oxygen demand (BOD) and pH were measured.

2.2. Yeast starter preparation

A yeast starter was prepared by adding one loopful of a 24 h-old slant culture into 250-ml Erlenmeyer flasks containing 50 ml of yeast and malt medium and incubating the flasks at 30 °C for 18 h on a rotary shaker operated at 150 rpm. After incubation, yeast cells were centrifuged at 6000*g*, 4 °C for 10 min, washed with sterile distilled water and collected by centrifugation. The washing procedure was done twice. The cells were suspended in sterile distilled water to make a final cell concentration of 10^6 cells/ml.

2.3. Yeast cultivation in flasks

For flask cultivations, the fermentations were carried out in 250-ml Erlenmeyer flasks containing 100 ml of sterile radish brine at various concentrations. Each flask was inoculated with the yeast starter of 5×10^6 CFU and incubated at 30 °C with an agitation rate of 150 rpm for 72 h. During incubation, samples were removed periodically for yeast growth and β-carotene determination. All shake flask experiments were carried out in triplicate. A suitable concentration of brine was selected to be used for batch cultivations in a bioreactor.

2.4. Yeast cultivation in a bioreactor

For batch cultivations in the bioreactor, the experiments were carried out in a 3-l stirred tank reactor (Biostat B, B. Braun Biotechnology International, Goettingen, Germany) containing 1.5 l of sterile diluted brine (obtained from 2.3) with adding 1 ml of propyl propylene as an antifoam agent. The brine was inoculated with the starter of 7.5×10^7 CFU and the bioreactor was controlled at 30 °C and kept pH constant at 6 with 60% dissolved oxygen for 24 h. Dissolved oxygen was automatically maintained at a set point by a controlling unit of the reactor using a two-stage cascade mode which regulated an aeration rate at the first stage and an agitation rate at the second stage. After the end of fermentation, the broth was removed for yeast growth and β-carotene analyses. The fermentations under various culture conditions were further studies according to the experimental design described below. All experiments were performed in duplicate.

2.5. Experimental design and statistical analysis

Three parameters; temperature (X_1), pH (X_2) and dissolved oxygen (X_3), were varied at three coded levels. Each variable was evaluated at a high (+1), a low (−1) and a central level (0) as detailed in Table 1. The variables were set up for 20 experiments (Table 1) consisting of 14 experimental runs and 6 additional runs at the center point level to check reproducibility by using a face-centered central composite design (Mongomery, 2001). Two experimental responses (yeast biomass and β-carotene production) from each experiment were recorded. Experimental data were analyzed by multiple regression using the Minitab software (Minitab Inc., State College, PA) and were used to develop a model according to the following quadratic polynomial equation:

$$Y = a + bX_1 + cX_2 + dX_3 + eX_1^2 + fX_2^2 + gX_3^2 + hX_1X_2 + iX_1X_3 + jX_2X_3, \quad (1)$$

where Y is the predicted response, X_1–X_3 are the considered variables and *a*–*j* are the coefficients obtained by the regression analysis of the data. The goodness of fit of the model was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA). Response surface plots were developed to indicate an optimum condition using the fitted quadratic polynomial equations obtained by holding one of the independent variables at a constant value and changing the levels of the other two variables.

2.6. Growth and β-carotene production under the optimum condition

Growth and β-carotene production of *R. glutinis* DM28 were studied in the bioreactor under optimum condition for 24 h to verify the predicted results.

2.7. Chemical analyses

Total soluble solid in the brine was analyzed by the APHA method (APHA, 1995). Total sugar was examined by phenol and sulfuric acid method (Multon, 1997). Total acidity expressed as lactic acid, nitrogen content, phospho-