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Improved β-carotene production of *Rhodotorula glutinis* in fermented radish brine by continuous cultivation

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ABSTRACT

Fermentation kinetics of growth and β-carotene production by *Rhodotorula glutinis* DM28 in batch and continuous cultures using fermented radish brine, a waste generated from fermented vegetable industry, as a cultivation medium were investigated. The suitable brine concentration for β-carotene production by *R. glutinis* DM28 was 30 g l^{-1}. Its growth and β-carotene production obtained by batch culture in shake flasks were 2.2 g l^{-1} and 87 μg l^{-1}, respectively, while, in a bioreactor were 2.6 g l^{-1} and 186 μg l^{-1}, respectively. Furthermore, its maximum growth rate and β-carotene productivity in continuous culture obtained at the dilution rate of 0.24 h^{-1} were 0.3 g l^{-1} h^{-1} and 19 μg l^{-1} h^{-1}, respectively, which were significantly higher than those in the batch. Therefore, improved growth rate and β-carotene productivity of *R. glutinis* in fermented radish brine could be accomplished by continuous cultivation.

1. Introduction

β-Carotene is an orange-yellow pigment commonly found in plant and animal tissues. The pigment belongs to carotenoid family and could be produced by numerous microorganisms such as *Sphingomonas* sp. (bacteria), *Dunaliella bardawil* (algae), *Blakeslea trispora* (fungi) and *Rhodotorula* spp. (yeast) [1–3]. β-Carotene is popularly used as an additive in food, feed, cosmetic and pharmaceutical products [4], because of its colorant with a benefit in anticancer and antioxidant activities [5].

Rhodotorula glutinis is one of β-carotene-producing microorganisms [6], which has been widely studied and has a potential for industrial production since it offers advantages over others in terms of high growth rate and the use of low-cost substrates. It can grow in several inexpensive 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 [4,7–13].

Fermented vegetables are indispensable diets in many Oriental countries including Thailand. Tons of fermented vegetables are annually produced for self-consumption in the country and for export. During the production process, a considerable amount of brine is generated as a waste containing high acid, salt and organic matters. The sauerkraut, fermented lettuce and fermented radish brines can be, for example, used as a substrate for β-carotene-producing yeast, *Rhodotorula* spp., in batch cultivations [14–16]. However, a low yield of β-carotene was produced by *R. glutinis* DM 28 in fermented radish brine under an optimum cultivation condition at 30 °C, a constant pH of 6 and 80% dissolved oxygen because of limited nutrients available in the brine [16].

Continuous culture has been employed to overcome substrate and product inhibitions or nutrient limitations in fermentation process. It also maintains microbial cell mass and product concentrations during the steady state resulting in increases in biomass and microbial productivity. This technique has been industrially applied for wastewater treatment, beer and vinegar production as examples.

To our knowledge, this report is the first to study the fermentation kinetics of *R. glutinis* in continuous culture compared to batch cultivation under a pH-uncontrolled condition using the radish brine as a sole substrate and to improve its growth and β-carotene production by continuous culture.

2. Materials and methods

2.1. Microorganism and media

R. glutinis DM28, a β-carotene-producing yeast isolated from fermented vegetable brine, was used [15,17]. It was stored at the microbial culture collection, Department of Microbiology, King Mongkut's University of Technology Thonburi, Thailand. The yeast was maintained on a yeast and malt extract (YM) slant (g l^{-1}: yeast

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Nomenclature

D	dilution rate (h^{-1})
P	β-carotene formation ($\mu g\,l^{-1}$)
q_P	specific β-carotene production rate ($\mu g\,g^{-1}\,h^{-1}$)
q_S	specific substrate consumption rate ($g\,g^{-1}\,h^{-1}$)
Q_P	β-carotene formation rate (productivity) ($\mu g\,l^{-1}\,h^{-1}$)
Q_S	substrate consumption rate ($g\,l^{-1}\,h^{-1}$)
Q_X	growth rate ($g\,l^{-1}\,h^{-1}$)
S	substrate consumption ($g\,l^{-1}$)
X	biomass concentration ($g\,l^{-1}$)
$Y_{P/S}$	β-carotene yield based on the substrate consumption ($\mu g\,g^{-1}$)
$Y_{X/S}$	growth yield based on the substrate consumption ($g\,g^{-1}$)

Greek letter

μ_{max}	maximum specific growth rate (h^{-1})

extract, 3; malt extract, 3; peptone, 5; glucose, 10; agar, 120) at 4 °C until used.

Fermented radish brine was supplied by a local pickle factory and kept at 4 °C until used. It contained ($g\,l^{-1}$): total sugar, 24; total acid, 3.7; nitrogen, 0.5; phosphorus, 7.5; NaCl 127, pH 4.4 [16].

2.2. Yeast inoculum preparation

A yeast inoculum was prepared by adding one loopful of a 24-h-old *R. glutinis* DM28 culture grown on a YM slant into 250-ml Erlenmeyer flasks containing 50 ml of YM medium. The flasks were incubated at 30 °C for 18 h on a shaker at 150 rev min^{-1}. After incubation, yeast cells were harvested 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^{-1} using a direct count method.

2.3. Batch fermentation in shake flasks

For flask cultivations, the experiments were carried out in 250-ml Erlenmeyer flasks containing 100 ml of sterile radish brine at various concentrations measured as total soluble solid (TSS) (10–90 $g\,l^{-1}$). Each flask was inoculated with 5×10^6 yeast cells and incubated at 30 °C with an agitation rate of 150 rev min^{-1} for 72 h. During incubation, samples were removed every 12 h for yeast growth and β-carotene determination including sugar, acid and pH analyses. Their kinetic parameters were also calculated. All shake flask experiments were carried out in triplicate. A suitable concentration of brine was selected for further experiments.

2.4. Batch cultivation in a bioreactor

The batch cultivation of *R. glutinis* DM28 was reinvestigated in a bioreactor to determine its fermentation kinetics and kinetic parameters compared to those obtained from the shake flasks. The fermentation was carried out in a 3-l stirred tank reactor (Biostat B, B. Braun Biotechnology International, Goettingen, Germany) containing 1.5 l of sterile diluted radish brine at a concentration obtained from the previous experiment with adding 1 ml of propyl propylene as an antifoam agent. The brine was inoculated with the starter of 7.5×10^7 cells. The bioreactor was automatically controlled at 30 °C, an air flow rate of 1 vvm and an agitation speed of 300 rev min^{-1} without pH control for 24 h. During fermentation, pH and dissolved oxygen in the broth were monitored and the broths were periodically removed for yeast growth, β-carotene, sugar and acid analyses. All experiments were performed in triplicate.

2.5. Continuous cultivation in a bioreactor

Continuous cultivation was carried out at the same culture condition as the batch cultivation. It started by feeding the brine at a suitable concentration with various dilution rates into the bioreactor and drawing broth with peristaltic pumps after the system reached a steady state (or after the batch culture of 24 h). During cultivation at each dilution rate, pH and dissolved oxygen in the broth were monitored and the broths were determined for yeast growth, β-carotene, sugar and acid after 10 medium volume changes. Their kinetic parameters were also calculated. All experiments were performed in triplicate.

2.6. Calculations of kinetic parameters

The kinetic parameters of the yeast growth and β-carotene production by both systems were calculated using the following formula:

Batch cultivation,

$$\mu_{max} = \frac{1}{X}\frac{dX}{dt} \tag{1}$$

where μ_{max} is the specific growth rate obtained during the exponential growth phase (h^{-1}), dX/dt is the growth rate (Q_X, $g\,l^{-1}\,h^{-1}$) and X is biomass concentration ($g\,l^{-1}$)

$$Y_{X/S} = \frac{\Delta X}{\Delta S} \tag{2}$$

where $Y_{X/S}$ is the growth yield based on the substrate consumption (g biomass g substrate $^{-1}$)

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \tag{3}$$

where $Y_{P/S}$ is the β-carotene yield based on the substrate consumption (μg β-carotene g substrate $^{-1}$)

$$q_S = \frac{1}{X}\frac{dS}{dt} \tag{4}$$

where q_S is the specific substrate consumption rate (g substrate g biomass^{-1} h^{-1}) and dS/dt is the substrate consumption rate (Q_S, $g\,l^{-1}\,h^{-1}$)

$$q_P = \frac{1}{X}\frac{dP}{dt} \tag{5}$$

where q_P is the specific β-carotene production rate (μg β-carotene g biomass^{-1} h^{-1}) and dP/dt is the β-carotene formation rate (Q_P, $\mu g\,l^{-1}\,h^{-1}$).

Continuous cultivation,

$$Q_X = XD \tag{6}$$

where Q_X is the growth rate ($g\,l^{-1}\,h^{-1}$), X is the biomass concentration for steady state ($g\,l^{-1}$) and D is the dilution rate (h^{-1})

$$Q_P = PD \tag{7}$$

where Q_P is the β-carotene productivity ($\mu g\,l^{-1}\,h^{-1}$) and P is the β-carotene concentration for steady state ($\mu g\,l^{-1}$)

$$Q_S = (S_0 - S)D \tag{8}$$