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Study of Growth Parameters for *Phaffia rhodozyma* Cultivated in Peat Hydrolysates

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ABSTRACT

The yeast *Phaffia rhodozyma*, known for its ability to synthesize carotenoids, was adapted and cultivated in liquid-phase media using peat hydrolysates as the main substrate source. The hydrolysates were prepared using high-pressure treatment at 185°C, without the addition of acid. The growth of the yeast was studied as a function of the pH, temperature, culture time, and agitation speed. The best conditions for the growth of the yeast were: a pH of 7, a temperature of 18°C, 5-d culture time, and 200-rpm agitation. Under those conditions, *P. rhodozyma* produced a concentration of 1279.82 μg carotenoids g^{-1} dry yeast, which compares well with other previously reported results.

Index Entries: Carotenoids; growth parameters; peat extracts; *Phaffia rhodozyma;* yeast.

INTRODUCTION

Phaffia rhodozyma (ATCC 24202, Miller et al.) is a recently discovered, carotenoid-producing yeast (*1*). It has been found to contain the carotenoid astaxanthin (*2*), which, when added to their diets, imparts a red coloration

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to the flesh of salmonids, shellfish (*3*), and poultry (*4*), thereby increasing their commercial value (*5*).

The cultivation of *P. rhodozyma* in various culture media has been described by several authors (*2,6–8*). Many studies conducted with *P. rhodozyma* have utilized synthetic culture media, and it has been reported that the presence of cellobiose and carotenoid precursors in these media enhances astaxanthin production (*9*).

Liquid extracts from peat produced by acid hydrolysis have been tested as a source of fermentation substrates (*10–14*). Since peat is a cellulosic material, its hydrolysis will result in the presence of cellobiose in the liquid component. Also, potential astaxanthin precursors, such as xanthophyll and carotenes, comprise about 2.6% of the peat bitumen fraction (*11*). Consequently, it is of interest to study the growth of *P. rhodozyma* in peat-based substrates. The present work reports the study of growth parameters for the adaptation and growth of the yeast *P. rhodozyma* cultivated in peat hydrolysates.

MATERIALS AND METHODS

Peat Hydrolysate

The peat hydrolysates used in this work were prepared using a pressure-temperature method (*15*). A pressure of 3000 psi and a temperature of 185°C for 2 min were the conditions used to produce the hydrolysates used in this work. Acid was not added to the peat before hydrolysis.

Culture

The yeast culture, *P. rhodozyma* ATCC 24202, used in this study was obtained from the American Type Culture Collection (Rockville, MD). It was maintained on Difco YM agar plates and transferred every month.

Inoculum Preparation

A loopful of yeast from the agar plates was aseptically inoculated into 20 mL of sterile Difco YM broth in a 125-mL Erlenmeyer flask and incubated in a Gyrotory water bath shaker (Model G76D, New Brunswick Scientific Co., Inc., Edison, NJ) at 22°C for 24 h. About 1 mL of this inoculum was used to inoculate the fermentation media.

Culture Conditions

The peat hydrolysates were diluted with equal volumes of water, centrifuged at $(1 \times 10^4) \times g$ for 30 min to remove suspended particles, and then filtered through a cellulose nitrate filter paper (pore size 0.45 μm, Sartorius

GmBH, Gottingen, Germany), in a Megaflow Membrane Filtration Apparatus (Model TM-100, New Brunswick Scientific Co. Inc., Edison, NJ). The filtration apparatus was fitted with a Watson-Marlow Peristaltic Pump (Model 502S, Watson-Marlow Ltd., Farmouth, Cornwall, England).

The total carbohydrate concentrations of the filtered peat hydrolysates were adjusted to 30 gL^{-1}, which was the concentration that produced the best yeast growth in preliminary tests. Hereafter in this work, total carbohydrate will be identified as "substrate." For the culture media, 50-mL portions of the filtered hydrolysate were adjusted to the required pH with 10*M* NaOH, supplemented with 0.68% (wv^{-1}) yeast nitrogen base (Difco Laboratories, Detroit, MI) and 0.60% (wv^{-1}) bactopeptone (Difco), and placed in 125-mL Erlenmeyer flasks. The medium was then autoclaved at $120 \pm 1°C$ for 20 min, cooled and inoculated, and then incubated in a Gyrotory water bath shaker for 120 h. After the experiments, the yeast biomass was harvested by centrifugation at $(1 \times 10^4) \times g$ for 30 min, washed twice with deionized water, and centrifuged again.

Growth Parameters

The following operating parameters were studied: initial pH (4, 5, 6, 7, 8), temperature (16, 18, 20, 22, 24°C), fermentation time (2, 3, 4, 5, 6, 7 d), and agitation speed (150, 200, 250, 300, 350 rpm).

Analytical Methods

The harvested cells were weighed wet, dried in a drying oven (Fisher Isotemp Model 106G, Fisher Scientific Co., Fairlawn, NJ) at 100°C for 18 h, and weighed again to calculate the dry biomass concentration. The total carbohydrate concentration of the substrate was determined by the method of Morris (*16*). The carotenoids were extracted from freeze-dried cells using a modified method of Gentles and Haard (*17*). The total carotenoid content was calculated using the formula of An et al. (*18*).

RESULTS AND DISCUSSION

Effect of pH

Figure 1 shows the yeast dry biomass concentration, yield (g of dry biomass produced $\times g^{-1}$ of dry substrate consumed) and efficiency (g of dry biomass produced $\times g^{-1}$ of initial dry substrate) as a function of the initial pH of the media. The best growth in terms of all three parameters was obtained at a pH of 7. Johnson and Lewis (*6*) reported that *P. rhodozyma* has optimal growth at pH 5. However, there are varied reports concerning the optimal pH for yeasts grown in peat hydrolysates. McLoughlin and Küster (*19*) and Chang (*20*) reported that high-pH peat hydrolysates

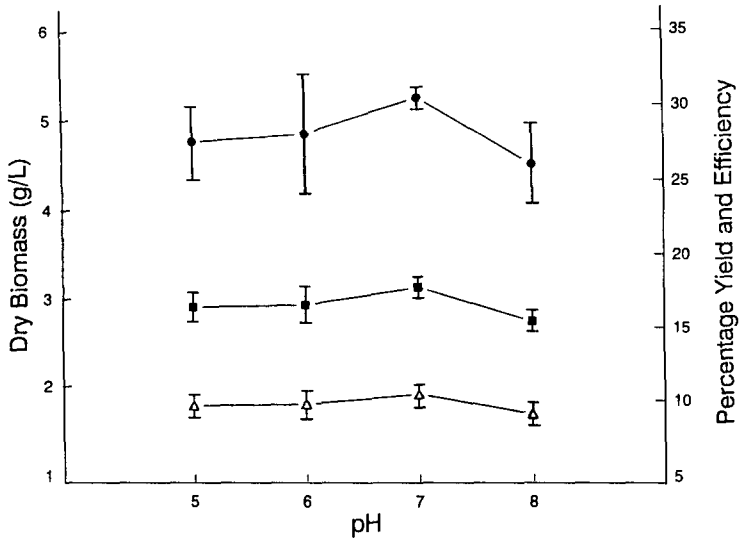

Fig. 1. Effect of pH on the growth of *Phaffia rhodozyma* in peat hydrolysate. Dry biomass: —■—, yield: —●—, efficiency: —△—.

sustain higher biomass yields of *Candida utilis* than low-pH hydrolysates. This they attributed to higher concentrations of humic substances in low-pH peat hydrolysates.

Effect of Incubation Temperature

Johnson and Lewis (*6*) reported that growth and pigment synthesis in *P. rhodozyma* are both optimal at 20–22°C. Figure 2 shows that the optimal growth temperature in this work was 18°C. There was no statistical difference ($P < 0.05$) in the yield values obtained at all temperatures investigated.

Effect of Fermentation Time

The effect of fermentation time on the growth of *P. rhodozyma* in peat hydrolysate is shown in Fig. 3. The highest dry biomass concentration, yield, and efficiency were obtained with 5 d of fermentation. In general, there was a gradual increase in growth from days 2 to 4, followed by an accelerated growth rate between days 4 and 5. There was a subsequent decrease in growth after day 5. There was a significant difference ($P > 0.05$) in all levels of measurement from each day of growth to the next. The total residual carbohydrate content of the culture media decreased sharply during the first 4 d of fermentation, after which the decrease became gradual, indicating that the culture had attained the stationary phase and that, possibly, most of the metabolizable carbohydrates in the substrate had been consumed.

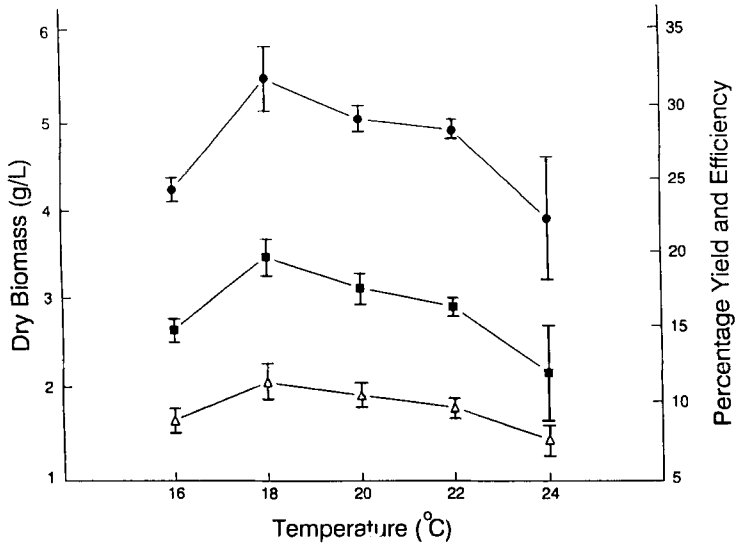

Fig. 2. Effect of incubation temperature on the growth of *Phaffia rhodozyma* in peat hydrolysate. Dry biomass: —■—, yield: —●—, efficiency: —△—.

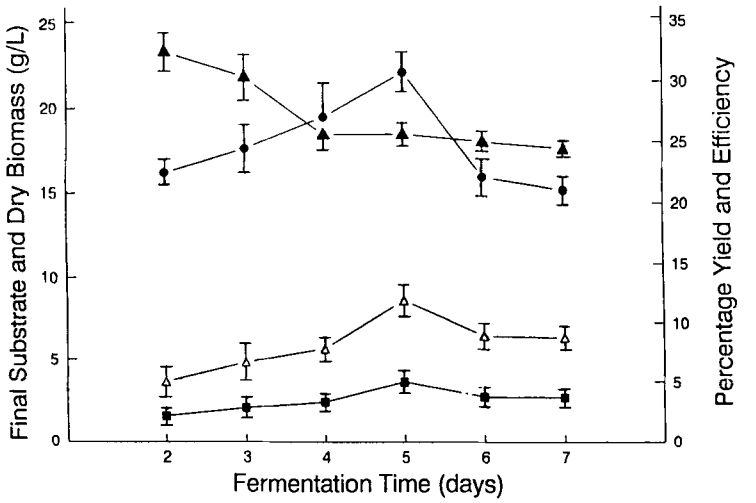

Fig. 3. Effect of fermentation time on the growth of *Phaffia rhodozyma* in peat hydrolysate. Dry biomass: —■—, yield: —●—, efficiency: —△—, final substrate: —▲—.

Effect of Agitation Speed

Previous works with *P. rhodozyma* have employed agitation speeds ranging from 150 to 600 rpm (*5,8,18,21,22*), but it has not been clearly stated which agitation speed was most suitable for the growth of the yeast. Figure 4 shows that, in our study, the growth parameters attained maximum values at 200 rpm.

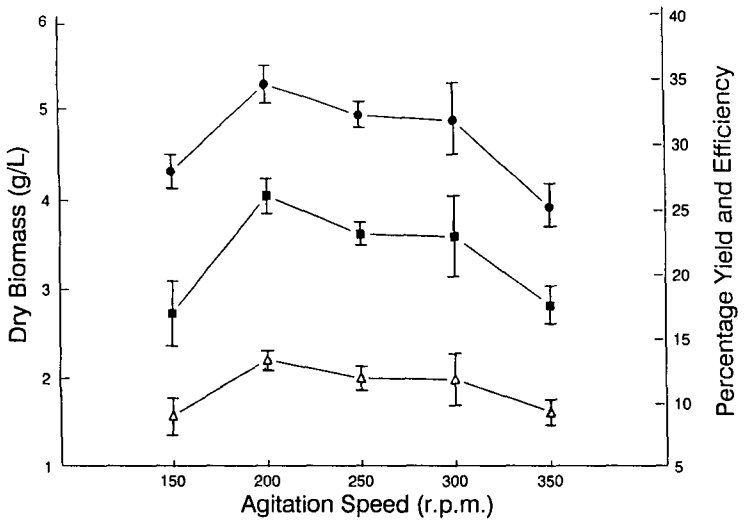

Fig. 4. Effect of agitation speed on the growth of *Phaffia rhodozyma* in peat hydrolysate. Dry biomass: —■—, yield: —●—, efficiency: —△—.

Carotenoid Content

The objective of the present study was to optimize the operating parameters for the yeast growth and not necessarily for the carotenoid content, for which they could differ. Further studies will concentrate on finding whether any difference exists in this regard. Nonetheless, the cultivation of the yeast under the growth conditions optimized in this work produced a concentration of carotenoids of 1279.82 ± 0.0 $\mu g\ g^{-1}$ dry yeast biomass. These values compare well with those reported elsewhere (*5,8,18*).

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