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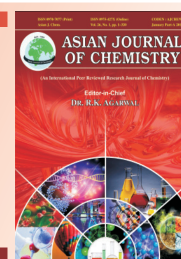

Effect of Addition of Activated Carbon on Lycopene Production of *Blakeslea trispora* in Submerge Cultivation†

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Lycopene production was increased when fine solid particle, activated carbon, was added to cultures of *Blakeslea trispora* (*B. trispora*) because of the enhanced dissolved oxygen concentrations. With 1 % (w/v) activated carbon added in the medium at zero day, lycopene production was 79 % higher than that of the control and the highest lycopene was 799 mg/L.

Keywords: Lycopene, *Blakeslea trispora*, Activated carbon.

INTRODUCTION

Lycopene is a red-coloured carotenoid with particular application as feed supplement and nutrient in human diets[1]. Because of its potential in alleviating chronic diseases such as certain cancers and coronary heart disease, it is considered to be a potent anti carcinogenic agent and antioxidant [2]. Natural sources of lycopene are fruits, vegetables and microorganisms. *Blakeslea trispora*, a Zygomycete mold, has been shown to be an effective producer of lycopene by introducing inhibitors of lycopene cyclase such as nicotine or pyridine into the medium[3,4].

B. trispora is an aerobic microorganism; sufficient supply of oxygen can increase both cell growth and carotenoid synthesis[5,6]. However, the high viscosity of the fermentation broth, the intertwined mycelial growth of *B. trispora* and the low solubility of oxygen in water result in a deficiency of the dissolved oxygen in the fermentation medium[7,8].How to increase the concentration of dissolved oxygen in the medium is crucial for the culture of *B. trispora.* Hemoglobin, perfluoro chemicals and hydrocarbons are used as oxygen-vectors in biotechnology[9,10]. Xu *et al.* used *n*-hexane and *n*-dodecane to increase production of lycopene and β-carotene by increasing the apparent solubility of oxygen in the medium[4].

Activated carbon is often used as a sorption agent for contaminated gas. It has a very high capacity of fixing components by means of van der Waals forces. It can apparently increase the absorption rate of natural gas in electrolytic aqueous solution[11]. In the past years, a number of researchers discussed the gas absorption mechanism in catalytic slurry reactors containing activated carbon which are considerably smaller than the gas-liquid film thickness and verified that the gas absorption rate can be enhanced considerably[12-15]. However, no studies have been reported on the gas absorption mechanism on aerobic microorganism submerged fermentation. In this report, the result of the addition of activated carbon on lycopene production by *B. trispora* in submerged cultivation was discussed so as to develop a new method to improve metabolism circumstances of aerobic microorganism cultivation.

EXPERIMENTAL

Pretreatment of carbon: Carbon was purchased from Kecheng Guanghua New Technology Co., Ltd. (Beijing, China). Carbon was screened with 200/60/16/5 mesh sieves and immersed in 0.5 % HCl overnight for being activated. Activated carbon was washed thoroughly to neutral with distilled water and dried in an oven at 40 ºC for 24 h.

Microorganism and culture medium: *B. trispora* ATCC 14271, mating type (+) and ATCC 14272, mating type (-), which were maintained on potato dextrose agar slants, were grown in the seed medium (starch 40 g/L, glucose 20 g/L,corn hydrolysate 50 g/L, KH_2PO_4 1.5 g/L,$MgSO_4$ 0.1 g/L, vitamin B_1 0.01 g/L, pH 6.4) at 28 ºC for 40 h in 250 mL flasks containing 50 mL medium. The cultures were used for the inoculation of the fermentation medium.

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General procedure

Fermentation conditions: The fermentation was carried out in 500 mL flasks containing 50 mL medium. The medium included 10 % inoculum which was a 1:4 (volume ratio) mixture of ATCC 14271 (+) and ATCC 14272 (-). The fermentation medium had the following composition (g /L): starch 20; soybean meal 40; corn hydrolyzate 30; KH_2PO_4 1; $MgSO_4$ 0.1; Vitamin B_1 0.01; pH 6.7. Activated carbon was added to the fermentation medium at the concentration of 1 % (w/v) or as otherwise stated. Before autoclaving the components of the medium, the fermentation medium was heated until dissolved completely. The flasks were incubated at 28 ºC in a rotary shaker incubator and the inhibitor, 5 mM nicotine, was added on the 2nd day for the lycopene production. Cultures were maintained for five days; the dry cell weight, lycopene contents were determined. All culture experiments and estimations were carried out in triplicate.

Detection method

Analytical methods: The cell mass was filtered through muslin, washed thoroughly with distilled water and dried in a vacuum drier at 45 ºC under 0.08 MPa vacuum for 48 h. The dried cells were weighed to determine the cell growth. To extract lycopene, the dried cells were cut into small pieces and homogenized with 25 mL petroleum ether until they became colourless. The petroleum ether phase containing lycopene was analyzed by high performance liquid chromatograph (HPLC) equipped with a Diamonsil C_{18} column (250 mm × 4.6 mm) at 28 ºC. The mobile phase contained acetonitrile and dichloromethane as a proportion of 75:25 (v/v) at 1.5 mL min/L. The injected volume was 10 µL. The absorbance of the lycopene was measured at 450 nm. Lycopene was identified by comparing with the retention time of the standard sample and quantitative analysis was performed by the single-point calibration process using an external standard (95 % lycopene). Dissolved oxygen concentration was measured by HQ Series Portable Meters(HACH, Catalog Number HQ 40d18).

RESULTS AND DISCUSSION

Effects of concentrations of activated carbon on lycopene production: A high dissolved oxygen concentration could promote the desaturation reactions and the lycopene synthesis in *B. trispora*. The result listed in Table-1 showed that when the activated carbon was 1 % (w/v) of the culture medium, the dissolved oxygen concentration reached a maximum value and then decreased slightly with a further increase of it. At these low solids concentrations, the enhancement of oxygen absorption was limited due to a rapid build-up of a layer of saturated particles close to the interface. The oxygen absorption by activated carbon particles released to the culture medium when *B. trispora* grew and decreased the high viscosity of fermentation broth. Activated carbon was washed away thoroughly when the cells were harvested on the 5th day for fermentation to ensure that there was no minus effect on the lycopene extraction.

Effects of size of activated carbon on lycopene production: Different sizes of activated carbon were added to the broth (screened by 200/60/16/5 mesh sieves) with a final concentration of 1 % (w/v). The results (Fig. 1) showed that when adding activated carbon screened by 60 mesh sieves in the fermentation culture the lycopene production was 75 % higher than that of the control. However, the larger ones almost had no effect. The best one is the one screened by 60 mesh sieves. The result was consistent with experiments previously reported[16].

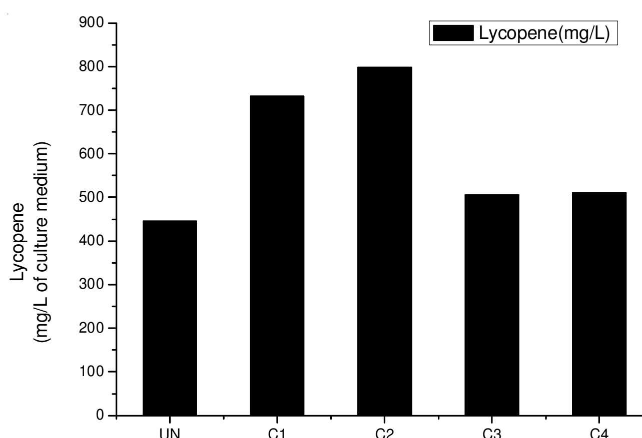

Fig. 1. Lycopene production by adding different size of activated carbon in the medium (UN = control, C1 = 200 mesh, C2 = 60 mesh, C3 = 16 mesh, C4 = 5 mesh)

Effects of adding time of activated carbon on lycopene production: The 60 mesh sieve screened activated carbon was added to the broth with a final concentration of 1 % (w/v) on day 0, 1, 2, 3 and 4, respectively. The biomass, lycopene production of *B. trispora* after five days' culture are shown in Table-2. For lycopene , the optimal adding time was at zero day. Although lycopene is a secondary metabolite that showed a sharp increase after the rate of cell growth begin to diminish[17], abundant oxygen in the initial growth stage was beneficial

TABLE-1
DRY CELL WEIGHT, LYCOPENE PRODUCTION AND RELATIVE DISSOLVED OXYGEN CONCENTRATION OF *B. trispora* WITH DIFFERENT CONCENTRATIONS OF ACTIVATED CARBONS (60 MESH) ADDED AT 0 DAY

Concentration of activated carbons (g/L)	Dry cell weight (g/L)	Lycopene content (mg/g)	Lycopene production (mg/L)	Relative dissolved oxygen concentration (%)
Control	52 ± 5	7.5 ± 0.6	446 ± 9	100
0.1	51 ± 6	10.2 ± 0.7	506 ± 10	100.5
0.5	51 ± 4	11.8 ± 0.7	609 ± 11	106.8
1.0	53 ± 5	17.1 ± 0.5	799 ± 13	114.4
2.0	54 ± 6	12.3 ± 0.8	592 ± 10	107.5
3.0	55 ± 5	10.6 ± 0.7	524 ± 12	97.6

Annotation: The cells were harvested on the 5th day and 5 mmol/L nicotine was added on the 2nd day. Values are means of triplicate ± standard deviation.

TABLE-2
DRY CELL WEIGHT, LYCOPENE PRODUCTION OF *B. trispora* WITH DIFFERENT ADDING TIME OF ACTIVATED CARBON (SCREENED BY 60 MESH SIEVE)

Adding time of activated carbon (day)	Dry cell weight (g/L)	Lycopene content (mg/g)	Lycopene production (mg/L)
Control	54 ± 3	7.5 ± 0.6	456 ± 5
0	55 ± 2	15.8 ± 1.0	764 ± 8
1	54 ± 6	14.5 ± 0.4	654 ± 9
2	53 ± 5	11.2 ± 0.7	537 ± 6
3	53 ± 6	10.6 ± 0.5	489 ± 7
4	55 ± 4	9.8 ± 0.8	459 ± 6

Annotation: The cells were harvested on the 5th day and 5 mmol/L nicotine was added on the 2nd day. Values are means of triplicate ± standard deviation.

to the synthesis of lycopene. With the addition of activated carbon at zero day, lycopene production by *B. trispora* reached 764 mg/L, which was 68 % higher than the control.

Conclusion

The results show some importance aspects of the effect of activated carbon on lycopene production. Activated carbon 1 % (w/v), added at zero day, produced high titers of lycopene due to the enhancement of dissolved oxygen concentration. A physical process for the high production of lycopene by mated cultures of *B. trispora* has been developed. Scale-up studies of this process are suggested as an economically attractive industrial process for the production of this in-demand nutraceutical carotenoid.

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