**Evaluation of nutrients and oxygen on the production of zeaxanthin by an Anarctic *Flavobacterium***

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**Highlights**

* *Flavobacterium frigidarium* is a promising source of zeaxanthin
* Oxygen supply enhanced the zeaxanthin production
* Evaluation of different concentrations of nutrients for the zeaxanthin production

**1. Introduction**

Carotenoids are the most diverse pigments present in nature. They are used in pharmaceutical, cosmetic and food industry as colorants and antioxidants. Traditional production of carotenoids is by chemical synthesis. However, the increasing negative perception of synthetic additives, demands an alternative such as the biotechnological production of these compounds. Bacterial production of carotenoids is still not competitive compared to chemical synthesis due to the high production cost and lower yields. Flavobacterium species are known as source of carotenoids, being zeaxanthin the principal product [1]. The oxygen supply to the culture is an important factor as oxygen is a precursor in the biochemical pathway for the conversion of β-carotene and β-cryptoxanthin in zeaxanthin. The aim of this work was to optimize the formulation of a media culture to produce zeaxanthin and scale-up the process in a laboratory scale fermenter using an Antarctic *Flavobacterium* sp.

**2. Methods**

A strain of *Flavobacterium frigidarium* [2] was used as source of carotenoids. The effects of three media components (peptone, yeast extract and NaCl) were studied by a factorial design 23 with four central points (Table 1). Media composition was as follows: peptone 2 or 12 g/L, yeast extract 2 or 12 g/L, NaCl 6 or 24 g/L, glucose 6 g/L, CaCl2 0.3 g/L, MgSO4.7H2O 3.4 g/L, urea 0.5 g/L and 400 µL/L of a micronutrient solution. The central point contained: peptone and yeast extract 7 g/L, and NaCl 15 g/L. The strain was cultured in 1-L Erlenmeyer flasks with 300 mL medium in an orbital shaker at 20°C and 200 rpm. The responses studied were zeaxanthin content (µg/gbiomass), zeaxanthin production (g/L), total carotenoid content (µg/gbiomass) and total carotenoid concentration (g/L). Biomass concentration was measured by OD at 600 nm. After 48 hours of growth, cells were harvested by centrifugation. Pellets were washed with distilled water, frozen at -80°C and lyophilized. Evaluation of dissolved oxygen influence was studied in a bioreactor Biostat A Plus (Sartorius) with 3 L of working volume at 20°C and pH 7. Dissolved oxygen was monitored and maintained above 20% of O2 saturation of the medium during the bioprocess. Biomass and carotenoid contents were monitored every 12 hours. After 72 hours of growth, cells were harvested and processed as explained previously. Carotenoid quantification was carried out by HPLC-DAD as previously described [2].

**3. Results and discussion**

The factorial design results are presented in Table 1. The highest zeaxanthin production was achieved in run 5 reaching (256 ± 19) µg/L. However, total carotenoid concentration was maximized in experimental conditions corresponding to the central point.

**Table 1.** Zeaxanthin and total carotenoid production in shaken flasks

|  |  |  |  |
| --- | --- | --- | --- |
| **Run** | **Factor (g/L)** | **Content (µg/g**biomass**)** | **Concentration (µg/L)** |
| **NaCl** | **Peptone** | **Yeast extract** | **Zeaxanthin** | **Total carotenoid** | **Zeaxanthin** | **Total carotenoid** |
| **1** | 6 | 2 | 2 | 47 ± 3 | 119 ± 6 | 112 ± 6 | 290 ± 13 |
| **2** | 6 | 2 | 12 | 27 ± 1 | 110 ± 2 | 111 ± 3 | 466 ± 6 |
| **3** | 6 | 12 | 2 | 43 ± 2 | 117 ± 5 | 132 ± 7 | 368 ±15 |
| **4** | 6 | 12 | 12 | 14 ± 1 | 64 ± 1 | 5 7 ± 1 | 273 ± 1 |
| **5** | 24 | 2 | 2 | 99 ± 7 | 172 ± 10 | 256 ± 19 | 455 ± 27 |
| **6** | 24 | 2 | 12 | 37 ± 3 | 109 ± 4 | 144 ± 10 | 435 ± 17 |
| **7** | 24 | 12 | 2 | 30 ± 2 | 92 ± 4 | 74 ± 6 | 233 ± 10 |
| **8** | 24 | 12 | 12 | 12 ± 1 | 56 ± 1 | 41 ± 1 | 192 ± 1 |
| **9** | 15 | 7 | 7 | 45 ± 2 | 121 ± 4 | 192 ± 9 | 521 ± 17 |
| **10** | 15 | 7 | 7 | 40 ± 1 | 110 ± 2 | 179 ± 2 | 495 ± 9 |
| **11** | 15 | 7 | 7 | 38 ± 2 | 111 ± 4 | 176 ± 9 | 509 ± 18 |
| **12** | 15 | 7 | 7 | 38 ± 2 | 107 ± 3 | 177 ± 7 | 501 ±12 |

As high levels of oxygen have been widely reported as enhancer of zeaxanthin production, both media were tested in fermenter to study the conversion of β-carotene and β-cryptoxanthin in zeaxanthin. In media 5, zeaxanthin and total carotenoid concentration were similar in shaken flasks and fermenter experiments, reaching in the latest (296 ± 5) µg/L and (443 ± 10) µg/L, respectively. It indicates that in this media, oxygen was not limiting for the production. However, in the central point media culture, total carotenoid production increased to (2358 ± 80) µg/L, with an almost complete conversion to zeaxanthin (2067 ± 70) µg/L. The oxygen supply had an important impact in the carotenoid production as it influences both biomass and zeaxanthin and carotenoid production. Biomass increased from (4.6 ± 0.1) g/L to (5.8 ± 0.1) g/L and zeaxanthin and carotenoid content resulted in (356 ± 12) µg/gbiomass and (407 ± 14) µg/ gbiomass respectively, 8- fold and 3- fold higher than in shaken flasks.

**4. Conclusions**

The results showed that the strain could increase the zeaxanthin concentration by 11-fold, showing the dependence of zeaxanthin production with media composition and oxygen supply.

**References**

[1] R. Carle and R. M. Schweiggert, Handbook on natural pigments in food and beverages: industrial applications, Eds Duxford, UK: Elsevier 2016.

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