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Microbial Carotenoids Production: Strains, Conditions, and Yield Affecting Factors

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***Abstract* – The research and development of carotenoid production has a long history, and interest in this group of pigments has not decreased to this day. Six existing carotenoids are considered industrially important: astaxanthin, β-carotene, lutein, zeaxanthin, canthaxanthin, and lycopene. These carotenoids have a wide range of applications and are used as additives in food and beverages, feed, nutraceuticals, pharmaceuticals, and cosmetics products due to their bioactive and pigmentation properties. Currently, the global pigment market is dominated by chemically synthesized carotenoids. Carotenoids derived from natural sources such as plants and microorganisms are not as popular or widespread. Currently, the market of natural carotenoids is mainly represented by microalgae *Haematococcus pluvialis, Dunaliella salina, Botryococcus braunii, fungus Blakeslea trispora*, yeast *Phaffia rhodozyma* and bacteria *Paracoccus carotinifaciens*. These microorganisms produce astaxanthin, β-carotene, canthaxanthin, and lycopene. Several yeast and bacteria species from *Rhodotorula*, *Sporobolomyces*, *Sporidiobolus*, *Gordonia*, and *Dietzia* genus can potentially become sources of carotenoids on an industrial scale, but available technologies still need improving. This paper reviews strategies for increasing the competitiveness of fungal and bacterial carotenoid production. Strategies such as selecting carotenogenic strains, using low-cost substrates, stimulating the synthesis of carotenoids by adding trace elements, TCA intermediates, NaCl, H_2O_2, light irradiation, and optimization of fermentation conditions such as pH, temperature and aeration are considered.**

***Keywords* – Agro-industrial wastes; astaxanthin; bacteria; β-carotene; canthaxanthin; lutein; lycopene; pigment; stress factors; yeast; zeaxanthin.**

1. INTRODUCTION

Carotenoids are natural or synthetic lipid-soluble pigments of yellow to orange-red colour, belonging to the class of tetraterpenes of 40 carbon atoms [1]. Carotenoids are classified into two groups. The first group is carotenes, such as α-carotene, β-carotene, γ-carotene, lycopene, and torulene. The second group is xanthophylls, such as astaxanthin, canthaxanthin, zeaxanthin, lutein, torularhodin, β-cryptoxanthin, and fucoxanthin [2]–[5]. Natural carotenoids are found in algae, plants, animals, fungi, cyanobacteria, bacteria [6], [7], and protists [8]. The primary function of carotenoids in plants, algae, and photosynthetic bacteria is photoprotection, i.e., the ability to quench reactive oxygen species formed during photosynthesis. Carotenoids also protect cells from other stresses, such as dehydration,

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osmotic pressure, high temperature, and high irradiance [9]. Carotenoids in non-photosynthetic plant parts such as fruits and flowers act as antioxidants, colour attractants, hormones, and aroma precursors [10]. In the case of microorganisms, the primary role of carotenoids is to protect cells against the harmful effects of oxidative and osmotic stress, light irradiation, nitrogen starvation, and low temperature [4], [11], [12]. In turn, animals (including humans) cannot synthesize carotenoids themselves. They must consume them through food to support physiological processes [13].

Due to their photoprotective, antioxidant, and colouring properties, carotenoids are widely applied in the food, feed, nutraceutical, cosmetic, and pharmaceutical industries [14]–[17]. In the food industry, carotenoids, mainly β-carotene and canthaxanthin, are used as colourant and antioxidant agents in fruit juices, beverages, pasta, candies, margarine, cheeses, milk, sausages, soft drinks, etc. [18], [19], [20]. In the feed industry, carotenoids, mainly astaxanthin and canthaxanthin, are used for pigmentation of the flesh and skin of salmon, trout, and ornamental fish [21], [22]. Canthaxanthin, zeaxanthin, and β-carotene used for pigmentation of shells and meat of crustaceans [23], [24], egg yolk of poultry, and for colouring ornamental bird plumage [25], [26]. In the pharmaceutical and nutraceutical industries, these carotenoids have found various uses due to their ability to interact with various systems in the human body [27]–[32]. Carotenoids, being antioxidants, reduce the negative impact of oxidative stress on human cells, which causes various chronic diseases such as cardiovascular diseases, osteoporosis, cancer, neurodegenerative diseases, etc. [31]. For example, lutein and zeaxanthin protect the eyes and skin from the harmful effects of solar radiation [27], and positively affect cognitive functions such as memory, learning rate, verbal fluency, etc. [28], [32]. Lycopene reduces the risk of developing various types of cancer, reduces cholesterol levels, activates an adaptive immune response, and as a result, protects against bacterial infection [29]. Astaxanthin has anti-inflammatory, anti-aging, immunostimulatory, anti-cancer, photoprotector, and anti-diabetic action [33], [34]. β-carotene is a dietary precursor of vitamin A, essential for eye health and immune system [30], [35].

The primary dietary sources of carotenes are fruits, vegetables, herbs, legumes, cereals, and vegetable oils [36]. Carrots, spinach, pumpkins, broccoli, sweet potatoes, tomatoes, apricots, and grapes have a high content of β-carotenes and lycopene [37]–[39]. The human diet's primary sources of xanthophylls like astaxanthin and canthaxanthin are animal products such as salmon, trout, crustaceans, and poultry eggs [40], [41]. For aquaculture farming, the situation is slightly different, since natural sources of astaxanthin for salmonids are microalgae and zooplankton, therefore, it is necessary to supplement aquaculture feed with astaxanthin [3], [40].

On an industrial scale, carotenoids are produced from natural sources such as plants, algae, and microorganisms or through chemical synthesis [17], [42]. Approximately 80–90 % of the global carotenoids market are synthetic carotenoids; the remaining small part comes from natural sources [21]. For example, natural carotenoids are produced from microalgae *Haematococcus pluvialis* [43] and *Dunaliella salina* [44], mould *Blakeslea trispora* (Xu *et al*., 2007) [45], yeast *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*) [46], bacteria *Paracoccus carotinifaciens* [6], from Marigold flower, and vegetables such as tomatoes, carrots, etc. [47]. The highest-demanded carotenoids on the world market are astaxanthin, canthaxanthin, zeaxanthin, lutein, β-carotene, and lycopene. Astaxanthin, β-carotene, and lutein account for 60 % of the market value [42], [48]. Carotenoid prices range from USD 200–2000 per kg for synthetic carotenoids and USD 350–7500 per kg for natural [49]. At the same time, the most expensive carotenoid is astaxanthin USD 2000–7500 per kg, depending on raw material for production [50]. The high percentage of synthetic carotenoids on the market is due

to their low production cost, higher stability, and easier emulsification than natural carotenoids. That facilitates their storage and application in various product matrices [19], [21]. In turn, the production of carotenoids from natural sources cannot compete with synthetic ones due to lower yields and slower growth of biomass that needs to be harvested, processed, and purified, all of which affect the cost of carotenoids and the end product [51], [52].

Despite the dominance of chemically synthesized carotenoids on the market, the demand for natural carotenoids is steadily increasing. This may be due to the growing awareness of the health benefits of carotenoids and the consumer's tendency to acquire natural products [48]. For example, the pinkish colour of salmon and trout flesh and the bright yellow colour of egg yolks are considered a sign of quality and are in demand among consumers [4], [53]. It is known that several chemically synthesized carotenoids are allergens, toxic, and carcinogenic [4], [19], [21]. In many countries, certain synthetic carotenoids are banned from use in food supplements and cosmetic products (astaxanthin, canthaxanthin, β-carotene) and banned or restricted in feed (canthaxanthin) [4], [54]. Moreover, studies show that synthetic carotenoids' antioxidant activity, anticancer activity, and bioavailability differ from natural ones. That is associated with differences in the stereometry and esterification of the molecules [55], [56]. For example, synthetic astaxanthin is 50 times less effective at quenching singlet oxygen, and 20 times less effective at scavenging free radicals than natural astaxanthin [54]. Therefore, researchers focus on improving biotechnological processes for the economically profitable production of natural carotenoids instead of synthetic ones [51].

Microbial carotenoids deserve special attention due to several advantages over other natural carotenoid producers. Microorganisms do not require a large area for cultivation, the yield does not depend on geographical factors and seasonality, as well as they are able to use agro-industrial by-products as a substrate for medium [14], [17]. Microbial carotenoids are more stable, less sensitive to light, more bioavailable, and have higher productivity than plant origin [5]. On the other hand, microbial biomass rich in carotenoids can be used directly as animal feed, but unlike plant biomass, it requires additional processing and purification [3]. Currently, microalgae are most often used to obtain carotenoids, since bacteria and fungi are inferior to them of yield. Using various strategies to improve carotenoid-producing strains, optimization of cultivation conditions, and using cheap feedstock raises the competitiveness of bacteria and yeast by increasing productivity and reducing production costs [37], [52].

Therefore, it is necessary to analyse each carotenoid-synthesizing microbial species to understand their potential for the biosynthesis of carotenoids. The review summarizes the data of experimental studies on producing industrially important carotenoids from fungi and bacteria: productivity and yield of carotenoids using agro-industrial by-products as feedstock, discusses the environmental parameters and fermentation conditions affecting carotenogenesis. The paper considers a strategy for increasing the competitiveness of microbial carotenoids – exposure to osmotic and oxidative stress, light irradiation, adding of precursors of the carotenogenesis pathway, and using two-stage fermentation.

2. Overview of Microbial Species Producing Industrially Important Carotenoids

As mentioned earlier, the most highly sought-after carotenoids in the global market are β-carotene, astaxanthin, canthaxanthin, zeaxanthin, lutein, and lycopene [42], [48]. Microbial carotenoids such as astaxanthin, β-carotene, and lutein are commercially produced mainly from microalgae. Although many technologies for producing carotenoids from bacteria and fungi are under research or development [57]. This is because microalgae cells are able to

accumulate more carotenoids than bacterial and yeast cells [58]. For example, the microalgae *Haematococcus pluvialis* can accumulate up to 0.8–4.8 % astaxanthin of dry biomass weight [59], [60], *Coelastrella* species are capable of producing canthaxanthin up to 4.75–15 % of dry weight [61], [62], and *Dunaliella salina* up to 6 % β-carotene and 5.4 % lutein simultaneously of the dry weight [63]. Whereas the yeast *Phaffia rhodozyma* (or teleomorphic state *Xanthophyllomyces dendrorhous*) generally contains 0.02–0.2 % astaxanthin [64]–[67], *Rhodotorula* sp. 0.02–0.07 % β-carotene [68]–[70], and bacteria *Dietzia maris* 0.1–2.8 % canthaxanthin [71]–[73] based on the dry weight of the biomass. However, bacteria and yeasts gradually becoming an alternative source of carotenoids due to strain improvement strategies, optimizing culture conditions, faster growth, higher biomass in liquid media, and other benefits [14], [58], [74]. For example, study [75] reported on 50-fold increase of the accumulation of astaxanthin by optimizing the cultivation condition of a chemically induced *Phaffia rhodozyma* mutant strain, with an impressively high biomass of about 100 g/L [75]. More strategies to improve carotenoid accumulation in bacteria, yeasts, and moulds are described in section 3.

The astaxanthin can be synthesized by yeasts *Phaffia rhodozyma* [46], [64], [66], *Rhodosporidium toruloides* [76], and *Sporidiobolus salmonicolor* [77], fungi *Mucor circinelloides* [78] and *Blakeslea trispora* [79], some bacteria species of *Gordonia* [80], [81] and *Paracoccus* [82], [83] genus, *Sphingomonas astaxanthinifaciens* [84], and some protists of *Schizochytrium* [85], [86], *Thraustochytrium* [87], and *Aurantiochytrium* [8] genus (see Table 1). These microorganisms are inferior in astaxanthin production to microalgae *Haematococcus pluvialis* with a productivity of 10.1–21.0 mg L^{-1} d^{-1} [59]. While the highest bacterial and fungal astaxanthin productivity rates reported in studies are 1.54 mg L^{-1} d^{-1} and 3.89–8.23 mg L^{-1} d^{-1} for *Schizochytrium* KH105 [85] and *Phaffia rhodozyma* [64], [67], [88], respectively. It is noteworthy that some microorganisms biosynthesize several types of carotenoids, e.g. *Gordonia alkanivorans* produces astaxanthin, lutein, canthaxanthin [80], and *Sporobolomyces ruberrimus* produces torularhodin, torulene, β-carotene and *γ*-carotene [89]. Wherein, proportions of the carotenoids will change under the influence of pH, dissolved oxygen presence, light intensity, medium composition, etc [80], [81], [90], [91]. While others produce basic carotenoids in higher quantities, e.g., *Phaffia rhodozyma* is capable of accumulating astaxanthin up to 83–87 % of total carotenoid content within its cells [92], for *Flavobacterium multivorum* it is about 93–96 % zeaxanthin content [93], [94] for *Dietzia natronolimnaea* this is canthatastaxanthin >89 % of total carotenoids content [95], [96].

According to the literature, canthaxanthin is found in the fungi *Cantharellus cinnabarinus* [97], as well as in the bacteria *Dietzia natronolimnaea* [96], [98] and *Dietzia maris* [72], [73] (see Table 1). The studies [71], [72] noted high concentrations of canthaxanthin in the strain *Dietzia maris* NIT-D, 1.65 % and 2.8 % of dry weight respectively, which is significantly higher than in many species of microalgae [26], [99]. Interestingly, *Dietzia maris* NIT-D, with a relatively low canthaxanthin content in its cells, is superior to the microalgae *Coelastrella* sp. D3-1 in productivity, although it can accumulate up to 15 % of this carotenoid in biomass due to the difference in biomass yield and cultivation duration. Thus, the productivity of canthaxanthin for *Dietzia maris* NIT-D is 24–105 mg L^{-1} d^{-1} under different cultivation conditions [71], [72], while for *Coelastrella* sp. D3-1 is 6.3 mg L^{-1} d^{-1} [62].

TABLE 1. QUALITATIVE AND QUANTITATIVE EVALUATION OF CAROTENOID-PRODUCING WILD-TYPE STRAINS OF FUNGI, BACTERIA AND PROTISTS

Microorganism	Carotenoid	DCW, g L^{-1}	CA, mg g^{-1}	CA, mg L^{-1}	CP, mg L^{-1} d^{-1}	FT	Carbon and nitrogen sources	Ref.
Yeast species								
Phaffia rhodozyma ATCC 74219	Astaxanthin	36.6	1.8	65.8	8.23	Batch	Sweet sorghum juice, yeast extract, urea, $(NH_4)_2SO_4$	[64]
Xanthophyllomyces dendrorhous DSMZ 5626	Astaxanthin	25.8	0.26	6.71	1.34	Flasks	Pectinase rapeseed meal hydrolysate, glycerol	[100]
		42	0.24	10.2	2.17	Batch		
Xanthophyllomyces dendrorhous DSMZ 5626	Astaxanthin	16.35	0.22	3.61	0.73	Batch	Glucose, glycerol, yeast extract, malt extract, $(NH_4)_2SO_4$	[65]
		18	0.27	4.8	1.15	Fed-batch		
Phaffia rhodozyma ENM5 1100	Astaxanthin	10.7	1.45	15.52	3.10	Flasks	Corn steep liquor, glucose, yeast extract, $(NH_4)_2SO_4$	[66]
Xanthophyllomyces dendrorhous	Astaxanthin	15.68	1.74	27.05	4.92	Batch	Glucose, yeast extract, KNO_3 $(NH_4)_2SO_4$	[88]
Phaffia rhodozyma SFAS-TZ08	Astaxanthin	45.2	0.43	19.47	3.89	Batch	Sweet potato juice, yeast extract	[67]
Rhodosporidium toruloides	Astaxanthin	n/d	n/d	0.93	0.23	Flasks	Glucose, peptone	[76]
Rhodotorula glutinis BCRC 22360	β-carotene	28.8	0.35	10.08	1.55	Batch	Crude glycerol, palm oil, yeast extract, $(NH_4)_2SO_4$	[68]
Rhodotorula mucilaginosa CC7688	Total carotenoids	16.1	0.23	3.73	0.93	Fed-batch	Sugar cane molasses, corn step liquor	[69]
Rhodotorula mucilaginosa MTCC-1403	β-carotene	7.33	0.72	5.28	1.5	Flasks	Pre-treated onion peels, mung bean husk	[70]
Sporidiobolus pararoseus JD-02	β-carotene	56.32	0.34	18.92	6.31	Fed-batch	Glucose, corn steep liquor	[101]
	Torulene		0.11	6.05	2.02			
Sporobolomyces roseus CCY 19-6-4	β-carotene	36.8	0.42	15.62	5.77	Fed-batch	Hydrolysed coffee ground, $(NH_4)_2SO_4$	[102]
Sporobolomyces ruberrimus H110	Total carotenoids	n/d	n/d	510	153	Batch	Crude glycerol, $(NH_4)_2SO_4$, yeast extract, peptone	[89]
Sporobolomyces ruberrimus ATCC 66500	Total carotenoids	33.50	2.97	99.49	n/d	Batch	Technical glycerol, $(NH_4)_2SO_4$, peptone, yeast extract	[103]
Mould species								
Mucor circinelloides CBS 277.49	β-carotene	9	0.7	6.3	3.15	Batch	Glucose, yeast extract, $C_4H_{12}N_2O_6$	[78]
Blakeslea trispora ATCC 14271	β-carotene	62	9.6	596	119.2	Flasks	Starch, soybean meal, corn hydrolysate	[45]

Blakeslea trispora ATCC 14271	Lycopene	57	9.3	533	106.6	Flasks	Starch, soybean meal, corn hydrolysate	[45]
Blakeslea trispora ATCC 14271	β-carotene	n/d	44.56	n/d	n/d	Batch	Glucose, corn steep liquor, yeast extract, casein acid hydrolysate, L-asparagine	[79]
	γ-carotene		38.62		n/d			
	Lycopene		1.83		n/d			
Blakeslea trispora NRRL 2895 (+), NRRL 2896 (-)	β-carotene	n/d	n/d	138	1.69	Flasks	Glucose, L-asparagine, yeast extract	[104]
Bacteria species								
Paracoccus sp. MBIC 01143	Astaxanthin	4.2	0.9	3.78	0.95	Flasks	Glycerol, soya peptone	[82]
Paracoccus aminophilus CRT1	Total carotenoids	n/d	0.63	n/d	n/d	Flasks	Pre-treated sawdust hydrolysate, yeast extract	[105]
Paracoccus kondratievae CRT2	Total carotenoids	n/d	0.76	n/d	n/d	Flasks	Pre-treated sawdust hydrolysate	[105]
Paracoccus zeaxanthinifaciens ATCC 21588	Zeaxanthin	n/d	0.75	n/d	n/d	Flasks	Glucose, yeast extract, peptone	[106]
Paracoccus zeaxanthinifaciens ATCC 21588	Zeaxanthin	n/d	n/d	11.63	3.88	Flasks	Glucose, yeast extract	[90]
Shingomonas natatoria KODA 19-6	Zeaxanthin	n/d	4.1	n/d	n/d	Flasks	Yeast extract, peptone	[107]
Sphingomonas astaxanthinifaciens NBRC102146	Total carotenoids	n/d	2.8	n/d	n/d	Flasks	Maltose, peptone, yeast extract, fish meat extract	[84]
Flavobacterium multivorum ATCC 55238	Zeaxanthin	n/d	n/d	10.65	5.81	Flasks	Glucose, yeast extract, peptone	[94]
Gordonia alkanivorans 1B	Total carotenoids	n/d	3.1	n/d	n/d	Flasks	Glucose, NH_4Cl	[81]
Rhodopseudomonas faecalis PA2	Total carotenoids	32	13	413	51.6	Flasks	Glutamic acid, yeast extract, $(NH_4)_2SO_4$	[108]
Dietzia maris NIT-D	Canthaxanthin	7.39	16.51	122.01	24.4	Flasks	Glucose, peptone, yeast extract	[71]
Dietzia maris NIT-D	Canthaxanthin	18.85	27.96	527.09	105.42	n/d	Coconut water, peptone, yeast extract	[72]
Dietzia maris AURCCBT01	Canthaxanthin	3.03	0.99	3.00	1.5	Flasks	Glucose, peptone, yeast extract	[73]
Dietzia natronolimnaea HS-1	Canthaxanthin	7.26	0.83	6.01	0.86	Batch	Beet molasses hydrolysate, yeast extract, peptone	[109]
Dietzia natronolimnaea HS-1	Canthaxanthin	9.11	0.98	8.92	1.27	Fed-batch	Glucose, peptone, yeast extract	[96]
Dietzia natronolimnaea HS-1	Canthaxanthin	17.79	0.82	14.63	2.09	Fed-batch	Beet molasses hydrolysate, yeast extract, peptone	[95]

Protist species								
Schizochytrium KH105	Astaxanthin	n/d	n/d	7.69	1.54	Batch	Glucose, distillery wastewater using barley	[85]
	β-carotene			4.64	0.93			
	Canthaxanthin			0.28	0.06			
Schizochytrium sp. S31	β-carotene	n/d	n/d	15.16	3.03	Fed-batch	Glycerol, yeast extract, $(NH_4)_2SO_4$, $C_5H_8NO_4Na$	[86]
Thraustochytrium striatum ATCC 24473	Astaxanthin	10	n/d	3.6	0.24	Fed-batch	Glucose, yeast extract, peptone	[87]

Note: DCW – dry biomass weight (grams per litre of medium); CA – carotenoids amount; mg g^{-1} – mg per g of DCW; mg L^{-1} – mg per L of medium; CP – carotenoids productivity; mg L^{-1} d^{-1} – mg per L of medium per day; FT – fermentation type; n/d – not defined.

Currently, commercial β-carotene is produced from the microalgae *Dunaliella salina* and mould *Blakeslea trispora* [4], [110], [111]. *Dunaliella salina is* reported to be an effective producer, accumulating up to 10 % β-carotene of the dry cells and 11.25 mg L^{-1} d^{-1} productivity [111]. Yeasts are able to synthesize β-carotene in more modest amounts, on average 0.02–0.04 % of the dry weight, however, they have a higher cell density in the fermentation medium than microalgae, which increases the total yield of carotenoid per litre of medium [69], [101], [112]. E.g., the biomass yield and β-carotene productivity for *Sporobolomyces roseus* CCY 19-6-4 and *Sporidiobolus pararoseus* JD-02 are 36.8 g L^{-1} and 5.77 mg L^{-1} d^{-1}, and 56.32 g L^{-1} and 6.31 mg L^{-1} d^{-1}, respectively [101], [102]. β-carotene is mainly synthesized by the red yeast *Rhodotorula* [69], [91], [112], [113], *Sporidiobolus* [101], [114], [115], *Sporobolomyces* [89], [116], *Cystofilobasidium capitatum* [113] and mould *Mucor circinelloides* [78]. The photosynthetic bacteria *Rhodopseudomonas faecalis* PA2 reportedly is capable of synthesizing β-carotene and lycopene [117], [118]. Depending on the microorganism β-carotene can be synthesized either as one of the final metabolites or as a primary metabolite precursor of astaxanthin, canthaxanthin, and zeaxanthin [2], [22]. *Sporobolomyces ruberrimus* is a producer of β-carotene, torularhodin and torulene. This species is able to accumulate 2.8 % of carotenoids in the total biomass [89], while biomass growth under optimal cultivation conditions may be relatively high around 34 g L^{-1} [103].

Lutein is another important carotenoid, which is not produced chemically due to low production yield [22]. It is obtained by extracting the biomass of the marigold flower *Tagetes erecta* L. [17]. The marigold flower contains about 1.5 % lutein and 0.5 % zeaxanthin (stereoisomer of lutein) per dry biomass weight [119]. This carotenoid is also present in microorganisms, mainly in microalgae. Microalgae *Dunaliella salina* [63], *Chlorella zofingiensis* [120], *Chlamydomonas planctogloea,* and *Desmodesmus protuberans* [99] are able to accumulate lutein in their cells up to concentrations of 5.36 %, 1.53 %, 0.4 %, and 0.74 % of the dry biomass, accordingly. The microalgae *Dunaliella salina* shows a relatively high yield and productivity of lutein, which is 155.2 mg L^{-1} and 9.7 mg L^{-1} d^{-1} [63]. It is reported that only a few of the bacteria, such as *Flavobacterium multivorum* [93], *Gordonia alkanivorans* [80], [81], and *Gordonia rubripertncta* [121] are able to synthesize lutein, however in small amounts.

On the other hand, many carotenoid-producing bacteria synthesize zeaxanthin (a stereoisomer of lutein), such as *Flavobacterium multivorum* [93], *Paracoccus aminophilus* [105], *Paracoccus zeaxanthinifaciens* [90], [106], *Paracoccus kondratievae* [105], *Shingomonas natatoria* [107] etc. Zeaxanthin accumulates in cells in similar concentrations of about 0.3–0.4 % of the dry weight in bacteria and microalgae. However, productivity is

higher for bacteria due to shorter cultivation time. E.g., as reported in the study [107], *Shingomonas natatoria* bacterium accumulated 0.4 % zeaxanthin of dry biomass weight over 4 days of cultivation in stirred flasks [107], while for microalgae *Synechococcus* sp. it takes 9 days to accumulate 0.23 % zeaxanthin of dry biomass and 21 days to accumulate 0.33 % when cultured in a photoreactor [122]. Moreover, *Flavobacterium multivorum* and *Paracoccus zeaxanthinifaciens* are capable of producing 5.81 mg L^{-1} d^{-1} [94] and 3.88 mg L^{-1} d^{-1} [90] of zeaxanthin, while for *Synechococcus* sp. it is 0.7 mg L^{-1} d^{-1} [122].

Besides *Phaffia rhodozyma, Blakeslea trispora,* and *Paracoccus* sp., which are already used industrially, the bacteria *Dietzia maris*, *Flavobacterium multivorum, Paracoccus zeaxanthinifaciens,* yeast *Sporidiobolus pararoseus*, *Sporobolomyces roseus*, *Sporobolomyces ruberrimus*, and *Cystofilobasidium capitatum* seem to be excellent candidates for carotenoid production based on productivity values (Table 1). Therefore, the relevance of the microorganism should be assessed not only by the amount of accumulated carotenoid in the cells, but also by the total weight of the biomass in the fermentation medium, and the carotenoid productivity. The use of inexpensive substrates for the cultivation of these microorganisms, the optimization of the culture medium and conditions, the manipulation of carotenoid synthesis, and the co-production of other valuable products simultaneously increase the competition of these microorganisms. The efficiency of extraction and purification of carotenoids from biomass undoubtedly plays an equally important role in economically profitable production [3], [65], [106], [123], but this aspect is beyond the scope of this review.

3. Selection and Optimization of the Fermentation Factors and Parameters

The content and composition of carotenoids in microorganism cells are not constant and vary depending on environmental conditions, the presence or absence of nutrients, and abiotic stresses [124]. Optimization of cultivation conditions and the composition of the fermentation medium can significantly increase the total amount of accumulated carotenoids in the biomass [67], [104], [125], and stimulate the accumulation of the target carotenoid by changing the ratio of the proportions of produced carotenoids [93]. Studies [104], [125] reported an increase in the content of β-carotene in the biomass of *Blakeslea trispora* by 42 % [104] and astaxanthin in *Phaffia rhodozyma* by 92 % [125] in the optimal medium compared to the non-optimized one. In another study [94], depending on the composition of the medium, *Flavobacterium multivorum* bacteria accumulated β-carotene, β-cryptoxanthin, and zeaxanthin in various proportions of the total mass from 3:4:93 to 82:7:11 [93]. To increase canthaxanthin production by *Dietzia maris* bacteria, glucose concentration, NaCl, and pH were key factors [73]. It is also important to consider that the type of fermentation used plays a role, as in [67] the biomass and amount of astaxanthin of the yeast *Phaffia rhodozyma* increased by 18.86 % and 32.5 %, respectively, during batch fermentation compared to shaking flask fermentation under similar conditions [67]. The fed-batch fermentation of *Rhodotorula mucilaginosa* increases total carotenoid amount approximately by 200 % compared to batch fermentation [69]. Table 1 summarizes the best results of the main microbial carotenoids obtained using agro-industrial by-products as substrate, applying stressors/synthesis stimulators, or optimizing cultivation conditions. The influence of these factors on the synthesis of major microbial carotenoids is described below.

3.1. Substrate selection and fermentation medium composition

The viability of the technology for the commercial production of microbial carotenoids directly depends on the cost of the process, which is influenced by many factors: the price of the fermentation medium, the cost of equipment and its operation (fermenters, biomass separating and processing, cleaning), the yield of carotenoids, productivity, and even the purchasing power of consumers, etc. [37], [52], [126]. One strategy to increase the competitiveness of the manufacturing process is to use inexpensive substrates as wastes and by-products [4], [7], [48], [52]. Therefore, microorganisms capable of using agricultural waste and industrial by-products as a nutrient source are being explored. Many studies on carotenoids use these products successfully (Table 1). Sweet sorghum juice [64], sweet potatoes juice [67], coconut water [72], corn steep liquor [66], [79], [101], molasses [69], [95], [109], cheese whey [98], rapeseed meal [127], soybean meal [45], crude glycerol [89], [113], [128], cooking oil [129], sawdust hydrolysate [105], wheat bran [7], [77], cassava residues [130], onion peels and mung bean husk [70], fruit and vegetable waste [131] are used for the cultivation of carotenoid-synthesizing microorganisms. At the same time, yeasts are able to grow in environments with a high carbon content (50–100 g L^{-1}) and utilize various sugars, as well as glycerol and oil. It results in reaching a high biomass and a good yield of carotenoids [70], [101], [114]. It is worth noting that, according to the assessment of many researchers for astaxanthin-producing yeast *Phaffia rhodozyma,* a sugar concentration of more than 50 g L^{-1} can have a negative impact on the biomass and the concentration of astaxanthin in cells [70], [127]. This is due to the occurrence of changes in metabolism, switching the process of respiration to alcoholic fermentation, which subsequently has an inhibitory effect [127]. However, it is possible to use batch or fed-batch fermentation, which will solve the potential problem with alcoholic fermentation and increase biomass yield [65]. Bacteria in comparison prefer lower sugar concentrations up to 20–30 g L^{-1} of medium and although the growth rate is higher, they are not as effective in biomass yield [73], [84], [95], [98], [107].

The source of C and N is important not only for the growth of biomass but also for the accumulation of carotenoids, however, the effectiveness of one substrate will depend on the preference of a particular microorganism strain [132], [133]. A study by [126] demonstrated that *Phaffia rhodozyma* preferred sucrose, fructose, and glucose, generating higher biomass and higher yield of astaxanthin per litre of a medium [126]. In another study, astaxanthin yield was comparable when using galactose, raffinose or glucose [132]. Many oleaginous yeasts successfully utilize oils and glycerol, which positively affects the yield of carotenoids [89], [113]. It was found that the use of crude glycerol resulted in a higher yield of carotenoids in the yeast *Sporobolomyces ruberrimus* compared to pure glycerol. This is due to the presence of fats in the raw glycerine after biodiesel production. This theory was confirmed by adding fatty acids to a medium containing pure glycerol. The researchers concluded that adding palmitic, stearic, oleic, and linoleic acids increased carotenoid productivity up to 2-fold [89]. These results are consistent with another study in which the addition of cottonseed oil increased the yield of carotenoids by 46 % in *Rhodotorula mucilaginosa* biomass [134]. Bacteria and yeast prefer yeast extract and peptone as an organic N source. Many studies show that these substrates provide maximum biomass and a high yield of carotenoids [67], [75], [90], [132]. However, to reduce the cost of the fermentation medium, inexpensive inorganic salts such as urea (CH_4N_2O), ammonium sulphate ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl), potassium nitrate (KNO_3) are also successfully used [80], [88], [89].

In addition to carbon and nitrogen sources, microorganisms need micro and macro elements that positively affect biomass and carotenoid accumulation. The study [93] reported on the stimulating effect of various salts on the synthesis of carotenoids in the cells of the bacterium *Flavobacterium multivorum*. With the addition of urea, sodium carbonate, and calcium chloride, the proportion of β-carotene increased without significantly inhibiting biomass. At the same time, in the presence of ammonium and magnesium salts, zeaxanthin was mainly synthesized, and growth inhibition was also observed [93]. Zinc, iron, copper, and magnesium have been reported to act as carotenoid inducers in some yeast species [127] and bacteria [82], [90], [96]. Fe^{3+} 30 ppm, Cu^{2+} 28.75 ppm and Zn^{2+} 27 ppm added to the medium had a significant effect on the synthesis of total carotenoids (9.7 mg/L) in Dietzia natronolimnaea in a fed-batch fermentation. At the same time, canthaxanthin also increased significantly (8.9 mg L^{-1}) [96]. In another study [108], a maximum total caronitoid yield up to 413 mg L^{-1} was achieved when 0.05 % Fe^{3+} was added to the medium for *Rhodopseudomonas faecalis* PA2, while 190 mg L^{-1} was obtained under non-optimized conditions. It is also interesting that Fe^{3+} supplementation reduced the cultivation time to reach maximum carotenoid values from 12 days to 8. This increased carotenoid productivity to 51.6 mg $L^{-1}d^{-1}$ compared to the initial results of 25 ml $L^{-1}d^{-1}$ in non-optimized conditions [108]. It was also reported that the addition of 1000 ppm vitamin A acetate to the medium significantly increased the synthesis of β-carotene in mould *Blakeslea trispora* at 828 mg L^{-1}, while subsequent addition of a piperidine inhibitor concentration of 500 ppm after 48 hours of cultivation increased the pool of lutein in relation to β-carotene by 775 and 67 mg L^{-1}, respectively [135]. Another study confirms the significant effect of methyl palmitate, ferrous sulphate, and pyridoxine hydrochloride on zeaxanthin synthesis by *Paracoccus zeaxanthinifaciens* [90].

As several studies show (Table 1), the cultivation of fungi on a medium based on agricultural plant residues or glycerine and/or waste oil along with the addition of ammonium sulphate can give a satisfactory yield of biomass and the productivity of the main carotenoid in comparison with the glucose-yeast extract-peptone medium. As for carotenoid-producing bacteria, glucose-yeast extract-peptone containing media are more often used. However, for some strains of bacteria, the alternative media may have glycerol for *Paracoccus* sp. [82], by-products for *Dietzia maris* [72], [95], [108] *Paracoccus aminophilus* [105], *Paracoccus kondratievae* [105] and ammonium chloride for *Gordonia alkanivorans* [80], [81]. Several studies [82], [90], [96], [127] confirm the importance of the trace elements such as iron, zinc and copper in significantly increasing the production of carotenoids in microbial cells.

3.2. Stimulators and stress factors supplements

Many studies have demonstrated that carotenoid synthesis is positively affected by the addition of tricarboxylic cycle acid (TCA) intermediates such as citrate, malate, succinate, pyruvate, fumarate, D–isocitrate and α–ketoglutarate, ferrous sulphate, etc. into the fermentation medium [82], [135], [136]. TCA is involved in forming the carbon skeleton of carotenoids and is also a source of energy used to synthesize carotenoids and fatty acids [74], [135]. When malic acid, isocitric acid and α-ketoglutarate were added to the medium at concentrations 6.02 mM, 6.2 mM, and 0.02 mM, respectively, zeaxanthin production in *Flavobacterium multivorum* biomass was reported to increase 6-fold [94]. Malate at 5 mM and ferrous sulphate at 1 mM addition increased astaxanthin yield in *Paracoccus* sp. 21 times, from 0.18 to 3.75 mg L^{-1} [82]. The presence of citrate 11.18 mM, malate 14.19 mM, glutamate 12.48 mM, and succinate 13.38 mM in the medium (pH 8) increased carotenoid yield in the anaerobic carotenogenic bacterium *Cellulosimicrobium* AZ to 28.86 mg L^{-1} compared to 2.4 mg L^{-1} in an unoptimized medium [137]. In another study [136],

canthaxanthin synthesis by *Dietzia natronolimnaea* increases with the addition of three intermediates at optimal concentrations: α-ketoglutarate 9.69 mM, oxaloacetate 8.68 mM, succinate 8.51 mM in a medium in fed-batch cultivation mode. As a result, reaching 13.2 mg L^{-1} canthaxanthin yield and 1.88 mg L^{-1} d^{-1} productivity compared to 8.97 mg L^{-1} and 1.28 mg L^{-1} d^{-1} under non-optimized conditions [136].

According to numerous studies, induced osmotic and oxidative stress is also an excellent stimulator of carotenoid production [12], [138], [139]. In a study on the impact of the osmatic stress caused by the addition of 5 % NaCl to the medium, on the synthesis of carotenoids by *Rhodotorula glutinis, Rhodotorula mucilaginosa,* and *Rhodotorula gracilis* – showed that there was an increase in total carotenoids and the proportion of β-carotene in all three yeast species [12]. On the other hand, oxidative stress caused by adding 5 mM – H_2O_2, increased the production of torulene in the biomass of all three strains, proportionally reducing the amount of β-carotene [12]. In another study, 2 % NaCl or 2 mM H_2O_2 adding to fermentation media resulted in a 2-fold increase in carotenoid production in *Rhodotorula glutinis*. In contrast, adding 2 % NaCl directly to the inoculant followed by adding 5 mM H_2O_2 to the fermentation medium increased the production of carotenoids by 3–4 fold [139]. Oxidative stress also affects the production of astaxanthin, with the addition of 10–20 mM H_2O_2 in the medium has a different effect on the accumulation of astaxanthin in *Xanthophyllomyces dendrorhous* cells depending on the growth phase of the yeast. The increase in astaxanthin was negligible with adding H_2O_2 to cells in the stationary growth phase. In contrast, adding H_2O_2 during the exponential growth phase increased the accumulation of astaxanthin by 60 % with little biomass inhibition. Also, the concentration of β-carotene in yeast cells decreased by adding 10 mM H_2O_2 after 0 and 24 hours of cultivation [140]. In another study [141] *Phaffia rhodozyma* yeast treatment by TiO_2 (0.5 g L^{-1}) doubled astaxanthin production within 72 h without apparent biomass inhibition [141].

Many studies have considered the effect of light illumination on increasing carotenoid production as a natural defense mechanism against photooxidative stress [2], [87]. Indeed, white light illumination increased the growth of biomass and the synthesis of carotenoids in the cells of the bacterium *Dietzia natronolimnaea* (canthaxanthin) [98] and *Gordonia alkanivorans* (lutein) [80], moulds *Mucor circinelloides* (β-carotene) [78], yeast *Xanthophyllomyces dendrorhous* (astaxanthin) [131], *Rhodosporidium toruloides* (β-carotene) [142], *Rhodotorula glutinis* (torularhodin) and *Rhodotorula mucilaginosa* (torularhodin) [12]. A study [78] shows that *Mucor circinelloides* is able to accumulate 2.2–2.7 times more β-carotene under continuous light compared to darkness, as a result, accumulating up to 0.7 mg g^{-1} of carotenoid in biomass for 48 hours of cultivation [78]. Cultivation of the genus *Rhodotorula* yeast under white light illumination increased the accumulation of torularhodin and torulene relative to β-carotene [12]. In another study, the authors [131] report that cultivation of *Xanthophyllomyces dendrorhous* under constant white light illumination showed a slight difference in the biomass and amount of astaxanthin compared to cultivation in the dark [131]. Thus, it has been established [80] that white light has a significant effect on the synthesis of lutein, canthaxanthin, and astaxanthin in the biomass of *Gordonia alkanivorans* in a glucose and sodium sulphate containing medium, while replacing these sources with others reduces the effect of light [80]. Interesting results were obtained by [81], it was reported that during cultivation in the dark, the synthesis of carotenoids in *Gordonia alkanivorans* cells ceased simultaneously with the depletion of glucose in the medium, while in the presence of light (400 and 3000 lm), the synthesis of carotenoids continued even in the complete absence of a carbon source. The authors note that this may be due to the ability of the strain to use the available reserves in the cells in the presence of light, which does not occur in the dark [81].

Interesting results are shown by a study [12], where stress factors such as 5 % NaCl, 5 mM H_2O_2, white light and low temperature were tested on three *Rhodotorula* strains and compared with a control (cultivation without stress). The most effective was a lower temperature – 20 °C instead of 28 °C. *Rhodotorula glutinis, Rhodotorula mucilaginosa*, *Rhodotorula gracilis* showed an increase in the productivity of total carotenoids from 0.96, 0.46 and 0.98 g L^{-1} d^{-1} to 1.22, 0.65 and 1.54 g L^{-1} d^{-1}, respectively. The remaining factors had less positive or negative effect on carotenoid synthesis in these three strains. Only white light irradiation significantly affected *Rhodotorula gracilis*, increasing carotenoid production from 0.98 g L^{-1} d^{-1} to 1.24 g L^{-1} d^{-1} [12].

It is worth noting that the effect of light on the synthesis of microbial carotenoids depends on the light source, light intensity, growth phase of the microorganism, and cultivation conditions

It can be concluded that TCA intermediates significantly affect carotenogenesis in bacteria, increasing the synthesis of zeaxanthin, astaxanthin, and canthaxanthin [74], [82], [94], [135], [136]. Due to the lack of scientific articles on using TCAs to increase carotenoid synthesis in fungi, it can be assumed that there is no positive effect for these microorganisms. Only one study reported no significant effect of TCA on β-carotene synthesis in *Blakeslea trispora* cells [135]. The opposite situation is with the use of NaCl and H_2O_2. The impact of these compounds on the synthesis of carotenoids has been described only for yeasts, and there are no studies on bacteria. Adding 2–5 % NaCl and combining 2 % NaCl with 2 % H_2O_2 increases carotenoid synthesis [12], [139]. However, higher concentrations of H_2O_2 as 5–10 mM reduce the synthesis of β-carotene in cells. Notably, for an astaxanthin-producing yeast strain, adding 10–20 mM H_2O_2 to the medium during the exponential growth phase leads to increased accumulation of astaxanthin [140]. In turn, the positive effect of light radiation on carotenogenesis is observed in bacteria and fungi. However, increasing the synthesis of microbial carotenoids depends on the light source, light intensity, growth phase of the microorganism, and medium composition and cultivation conditions. Therefore, light radiation appears to be a less critical factor influencing carotenoid synthesis in bacteria and yeast.

3.3. pH and temperature

It is known that pH and temperature are essential factors for successfully cultivating microorganisms, and their optimal values can increase the production of certain cellular products [64]. The best pH and temperature for carotenoid production varies depending on the type of microorganism and the type of carotenoid. *Phaffia rhodozyma* has a wide pH range at which it can grow and produce carotenoids, with many studies reporting optimal pH values for astaxanthin production to be between 4 and 7.5, at 20–22 °C [64], [67], [130], [132]. The work [88] reported that the maximum biomass growth of Phaffia rhodozyma was observed at pH 6. In contrast, a decrease of pH to 5 and 4 decreased the yield of biomass but increased the yield of astaxanthin. The productivity of astaxanthin was 3.22, 4.36, and 3.78 mg/L/d under controlled batch fermentation conditions of pH 4, 5, and 6, respectively. Improved astaxanthin productivity results of 5.41 mg/L/d and 4.57 mg/L/d were achieved by switching the initial controlled pH of 6 to pH 5 and 4 at 80 hours of fermentation, respectively [88]. Considering this pH control and splitting the fermentation process into 2 steps, biomass accumulation and astaxanthin synthesis is a good strategy to optimize carotenoid production [2], [88]. A similar situation is observed for *Paracoccus sp.*, when a lower temperature of 20 °C resulted in a 39 % increase in astaxanthin, while the optimum temperature for biomass

was 25 °C. However, the improved productivity of 0.058 mg L^{-1} d^{-1} was at 20 °C compared to 0.4 mg L^{-1} d^{-1} at 25 °C [82].

The optimum pH and temperature for carotenoid production varies greatly for red yeast, natural producers of β-carotene, torularhodin, and torulene. For example, work [101] reported that *Sporidiobolus pararoseus* has good biomass growth at pH 4–8 and 25–35 °C, which is optimal for both carotenoids and biomass at pH 6 and 28 °C. At the same time, these cultivation factors did not affect the ratio of the proportions of carotenoids [101]. In another study [12] 3 species of yeast *Rhodotorula* synthesized a more incredible amount of total carotenoids at a temperature of 20 °C compared to cultivation at the optimum temperature for the biomass of these species of 28 °C. The concentration of carotenoids in the biomass increased by 19 %, 46 %, and 57 % for *Rhodotorula glutinis*, *Rhodotorula mucilaginosa,* and *Rhodotorula gracilis* when cultivated at 20 °C, respectively. Additionally, there was an increase in the proportion of β-carotene and a decrease in torulene and torularhodin [12]. In other studies, the optimum temperature and pH for carotenoid production from *Rhodotorula mucilaginosa* based on various sugars from agro-industrial waste were reported to be pH 6.1 and 25.8 °C [70], pH 7.0 and 30 °C [134], and pH 5 and 22–28 ° C [143]. Since temperature and pH are important triggers for enzymatic reactions [107], it is possible that when using different substrates that require different enzymatic conversion, the optimal pH and temperature will vary even within the same species.

According to multiple sources, the cultivation of carotenoid-producing bacteria occurs at a temperature of 25–37 °C and a pH of 5.5–7.5 [82], [84], [95], [107], [136], [144]. It is reported that the optimal temperature for the release of *Paracoccus sp.* biomass is 20–25 °C, while a lower temperature of 20°C stimulated the release of astaxanthin by 39 % compared to 25 °C [82]. In another study, the maximum yield of zeaxanthin from *Shingomonas natatoria* was obtained at 30 °C. In comparison, the optimal temperature for growth was 35 °C, although culturing bacteria at 25 °C gave average readings of biomass and zeaxanthin. As a result, carotenoid productivity was 0.06 mg L^{-1} d^{-1}, 0.18 mg L^{-1} d^{-1}, and 0.02 mg L^{-1} d^{-1} at 25 °C, 30 °C, and 35 °C, respectively [107]. Cultivation of *Dietzia natronolimnaea* in the temperature range from 13 to 31 °C had no significant effect on biomass. The biomass maximum slowly increased from 5.91 to 6.09 g L^{-1} as the temperature increased to 31 °C and fell more rapidly at 34 and 40 °C, 5.76 and 1.94 g L^{-1}, respectively. However, temperature strongly influenced the growth rate of bacteria; at 31 °C, the maximum biomass was reached at 168 hours of fermentation, while at 13 °C at 340 hours. Interestingly, the maximum yield of canthaxanthin was observed at 31 °C, while the maximum yield of total carotenoids was at the lowest fermentation temperature. pH 7 proved optimal for biomass, total carotenoids, and canthaxanthin products of *Dietzia natronolimnaea* [98].

Temperature and pH of the environment are the basic factors influencing the synthesis of microbial carotenoids. According to the literature, the optimal temperature and pH for synthesizing carotenoids either corresponds to the optimal conditions for biomass growth or is lower than these readings. Therefore, after optimizing the environment and conditions for the target strain and type of carotenoid, it is necessary to experiment with lower temperatures and pH values relative to the optimal one.

3.4. Aeration

Adequate aeration of the fermentation medium is essential for the growth and synthesis of carotenoids in aerobic microorganisms [37], [45], [65], [70], [145]. The requirement for oxygen during fermentation can be fulfilled by mixing the flasks on an orbital shaker and adjusting the working volume of the medium in the flasks, or by combining the supply of

oxygen and mixing the medium in a bioreactor [66], [70]. Studies show that under the same preassigned fermentation conditions, the biomass and yield of carotenoids were significantly higher in the bioreactor compared to shake flasks [65]–[67], [127]. For example, *Phaffia rhodozyma* cultivation in a 5 L bioreactor increased in biomass and astaxanthin yield by 18.9 % and 32.5 % compared to shake flask fermentation [67]. These results are consistent with other work (see Table 1). Scientific works report high levels of astaxanthin production with high aeration of the medium during the fermentation process, in the ranges of 40–80 % dissolved oxygen (DO) saturation [64], [146] and/or stirring speed of 300–600 rpm [65], [67], [127]. Increasing DO from 20 % to 80 % is reported to increase biomass and astaxanthin production in *Xanthophyllomyces dendrorhous*. Moreover, high dissolved oxygen levels may interfere with yeast growth inhibition associated with high glucose concentrations in the medium [65]. Also, the regulation of DO saturation affects carotenoid ratios in β-carotene, torulene, and torularhodin-producing yeast [70], [101]. When *Sporidiobolus pararoseus* was cultivated at 5 % DO, carotene was 60.56 % and torulene 18.02 % of the total amount of carotenoids, while with an increase in DO concentration, the proportions of carotenoids changed towards an increase in torulene. At 40 % DO it resulted in 33.72 % carotene and 50.12 % torulene concentration of the total produced carotenoids [101]. To enrich the fermentative medium with oxygen when growing the mould *Blakeslea trispora*, additional measures are required due to the high viscosity of the medium created by the mycelium biomass. Adding oxygen-vectors and surfactants to the fermentation medium positively affects the production of biomass and carotenoids [45], [104]. For example, adding n-hexane and n-dodecane at 1 % v/v increased the concentration of lycopene and β-carotene in cells, and also positively affected biomass. The maximum yield of these carotenoids was obtained by adding 1 % v/v to the medium n-dodecane together with 0.1 % w/v surfactant Span 20 [45]. In another study, adding 0.2 % w/v surfactant Span 20 significantly increased β-carotene production (229 %) by *Blakeslea trispora,* which amounted to 318 mg/L of medium [135].

It can be concluded that astaxanthin-producing yeasts require a high concentration of 40–80 % DO in the environment for high yield of biomass and astaxanthin. For yeast producing a carotenoid mix, the concentration of dissolved oxygen in the medium will affect the ratio of these carotenoids in the cells. Therefore, it is necessary to experimentally select the required dissolved oxygen concentration for the target carotenoid. There is no data on the effect of dissolved oxygen concentration on the synthesis of bacterial carotenoids. Improving the yield of biomass and carotenoids in mould is carried out through oxygen-vectors, which increase the access of oxygen to medium with increased viscosity. Surfactants, n-hexane and n-dodecane can be applied in small quantities to improve the synthesis of lycopene and carotene by *Blakeslea trispora*.

4. CONCLUSIONS

Studies show that maximum values of carotenoids are achieved under multifactorial cultivation conditions aimed at two main tasks. The first is to achieve high biomass, which in the case of yeasts and moulds is an advantage over bacteria and microalgae. Even at relatively low concentrations of carotenoids in cells, the yield of carotenoids at high biomass concentrations becomes competitive with that of microalgae. For this purpose, it is necessary to optimize the cultivation conditions for each particular strain: the composition of the nutrient medium, temperature, pH, adequate aeration, nutrition, and the addition of growth essential elements must be checked. The second task is to increase the synthesis of total

carotenoids or a specific target carotenoid in the case of a microorganism synthesizing a mix of carotenoids. This also requires optimization of cultivation factors as in the case of the first task. However, it is important to understand and distinguish that the parameters of these factors will be different. It is worth noting that bacteria have a more diverse composition of carotenoids compared to yeast and show an excellent improvement in carotenoid synthesis while optimizing the fermentation process aimed at carotenogenesis.

Microbial carotenoid synthesis can be improved using the following strategies:

- Addition of TCA intermediates: citrate or isocitric acid, malate or malic acid, succinate, α-ketoglutarate, glutamate, oxaloacetate to the medium for carotenoid-synthesizing bacteria.
- Addition of NaCl, H_2O_2, TiO_2, etc. to the medium for carotenoid-synthesizing yeast.
- Experimentally determine suitable cultivation conditions for the target strain and carotenoid type by applying lower pH values and temperatures relative to the optimum for biomass yield.
- Maintain a high concentration of dissolved oxygen in the environment to improve astaxanthin production and lower values for carotene production.
- Use oxygen vectors to improve aeration for carotenoids yield by mould.

In the future, it is important to expand knowledge and define the influence of the growth phase of microorganisms on the result of using factors that improve carotenogenesis. In this way, achieving high productivity of microbial carotenoids will be possible. Because optimal parameters for the synthesis of carotenoids are often inhibitory for biomass growth, applying a two–stage fermentation strategy is necessary. Where high biomass is achieved under optimal cultivation conditions at the first stage, the factors that have a greater influence on the synthesis of carotenoids for a particular strain are switched to the required values. The application of this two-stage fermentation strategy in combination with other strategies such as the use of inexpensive agro-industrial waste, the co–production of high value–added components, the use of efficient extraction methods, the improvement of strains by mutagenesis or genetic engineering, etc. allows us to consider bacteria, yeast, and mould as a worthy alternative to microalgae.

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