Journal of Applied Phycology (2018) 30:3009–3015
https://doi.org/10.1007/s10811-018-1553-8

8TH ASIAN PACIFIC PHYCOLOGICAL FORUM

Effects of *Xanthophyllomyces dendrorhous* on cell growth, lipid, and astaxanthin production of *Chromochloris zofingiensis* by mixed culture strategy

Xueya Jiang[1] · Lu Liu[1] · Junhui Chen[1] · Dong Wei[1]

Received: 31 January 2018 / Revised and accepted: 17 June 2018 / Published online: 26 June 2018

Abstract
The alga *Chromochloris zofingiensis* and the yeast *Xanthophyllomyces dendrorhous* are typical microorganisms which can accumulate high-value astaxanthin and lipid simultaneously. This study investigated the synergistic effects of *X. dendrorhous* on the cell growth, lipid, and astaxanthin production of *C. zofingiensis* by a mixed culture approach. Compared to the pure culture of *C. zofingiensis*, enhanced lipid and astaxanthin production were obtained in the mixed culture. The maximum astaxanthin and lipid yield achieved in the mixed culture with the ratio of 3:1 (algae to yeast) were 5.50 mg L^{-1} and 2.37 g L^{-1}, respectively, which were 1.10- and 2.72-fold that of *C. zofingiensis* monoculture. Additionally, lipid obtained from the mixed culture had a plant oil-like fatty acid composition. This study provides a new insight into the integration of natural astaxanthin production with microbial lipid.

Keywords Lipid · Astaxanthin · Mixed culture · Yeast · Alga

Introduction

Astaxanthin, which has a broad spectrum of potential applications (e.g., functional food additives, cosmetics, and pharmaceutics), has attracted much attention because of its anticancer and antioxidant properties (Dong and Zhao 2004; Ambati et al. 2014; Liu et al. 2014). Synthetic astaxanthin is a large proportion of the commercial supply. Compared with synthetic astaxanthin which is a mixture of 3*S*,3'*S*, 3*R*,3'*S*, and 3*R*,3'*R* stereoisomers, natural astaxanthin is in the 3*S*,3'*S* form, which provides a higher pigmentation than other forms (Osterlie et al. 1999). In addition, natural astaxanthin is much more stable than synthetic astaxanthin. However, the production process of natural astaxanthin from the carapaces of certain crustaceans such as krill is still too costly to make it economically competitive with synthetic production.

Recently, the production of natural astaxanthin by microorganism, such as microalgae, yeast, and bacteria, has attracted considerable interests. As the main commercial producer of microalgae-based natural astaxanthin, *Haematococcus pluvialis* can accumulate astaxanthin with a high content using CO_2 in photosynthesis under environmentally stressed conditions such as high irradiance and deficiency of nitrogen (Borowitzka et al. 1991; Boussiba 2000). However, its slow growth rate, low biomass concentration, and dependence on high light for astaxanthin accumulation largely limit its industrial application (Ip et al. 2004; Zhang et al. 2017b). *Chromochloris zofingiensis* (previously known as *Chlorella zofingiensis)* has recently attracted attention due to its ease of growing under various conditions (e.g., autotrophic, mixotrophic, and heterotrophic culture conditions) with fast growth and high cell density (Kim et al. 2016). Compared to *H. pluvialis*, *C. zofingiensis* is less light-dependent and can be better controlled; hence, it is potentially more economical for commercial astaxanthin production (Liu et al. 2014).

The red pigmented yeast *Xanthophyllomyces dendrorhous* also has great industrial potential for the production of natural astaxanthin due to its short growth cycle. However, compared to *H. pluvialis*, it contains less astaxanthin. Both *C. zofingiensis* and *X. dendrorhous* have been proposed as promising producers of algal fatty acids and high-value pigments

Xueya Jiang and Lu Liu contributed equally to the work and should be regarded as co-first authors

✉ Dong Wei
fewd304@scut.edu.cn

[1] School of Food Sciences and Engineering, South China University of Technology, Wushan Rd. 381, Guangzhou 510641, People's Republic of China

(Dominguez-Bocanegra et al. 2007; Sun et al. 2008). The correlations between lipid accumulation and the synthesis of fat soluble pigment under stress conditions have been widely reported (Zhekisheva et al. 2002; Cheirsilp et al. 2011). Therefore, the integration of natural astaxanthin production with other high-value products by those two species could provide promising approaches for profitable production of algal biomass.

Mixed culture, being common in natural systems, is cultivation of two or more species at the same system (Qin et al. 2018). Recently, mixed cultures of yeasts and microalgae have been studied in various aspects including waste treatment, enzyme regeneration, and fine chemical production (Santos and Reis 2014; Zhang et al. 2017a; Liu et al. 2018). It was demonstrated that there were synergistic effects between algae and yeast in a mixed culture system on pH adjustment, O_2/CO_2 balance, and substance exchange, which eventually led to higher productivities (Cai et al. 2007; Yen et al. 2015). Experiment has been carried out only in an autotrophic culture system of microalgae to enhance the CO_2 fixation and astaxanthin production using the mixed culture of *H. pluvialis* and *X. dendrorhous* (Dong and Zhao 2004). There is little information available regarding the effects of yeast on the natural astaxanthin production integrated with lipid accumulation in a mixotrophic culture system. Thus, herein for the first time, two different astaxanthin and lipid-producing strains, *C. zofingiensis* and *X. dendrorhous*, were co-cultivated with the supplement of an organic carbon source. The aim of this study is to give a new insight into the production of valuable microbial metabolites by mixed culture.

Materials and methods

Strains

Chromochloris zofingiensis (ATCC 30412) was purchased from American Type Culture Collection (ATCC) and maintained at 4 °C in Bristol's medium containing 0.75 g L^{-1} $NaNO_3$, 0.175 g L^{-1} KH_2PO_4, 0.075 g L^{-1} K_2HPO_4, 0.075 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.025 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.025 g L^{-1} NaCl, 5 mg L^{-1} $FeCl_3{\cdot}6H_2O$, 0.287 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.169 mg L^{-1} $MnSO_4{\cdot}H_2O$, 0.061 mg L^{-1} H_3BO_3, 0.0025 mg L^{-1} $CuSO_4{\cdot}5H_2O$, and 0.00124 mg L^{-1} $(NH4)_6Mo_{24}{\cdot}7H_2O$. *Xanthophyllomyces dendrorhous* (AS2.1557) was purchased from Guangdong Microbiology Culture Center and maintained at 4 °C on YM (Mold and Yeast Chromogenic) medium which consisted of 10 g L^{-1} glucose, 5.0 g L^{-1} Bacto Peptone, 3.0 g L^{-1} malt extract, 3.0 g L^{-1} yeast extract, and 20 g L^{-1} agar. Bristol's medium and YM were used for the seed culture of *C. zofingiensis* and *X. dendrorhous*, respectively.

Culture medium and conditions

BBM medium (Bold's basal medium) (Nichols and Bold 1965) with glucose as carbon source and urea substituting for $NaNO_3$ at an equal N molar ratio was used for the batch culture; the initial C/N ratio was adjusted to 180. The seed cells were inoculated into flasks containing 100-mL culture medium of *C. zofingiensis* or a mixture of *X. dendrorhous* and *C. zofingiensis*. Different *C. zofingiensis*/*X. dendrorhous* inoculum ratios (1:0, 1:1, 2:1, 3:1) were co-cultured to investigate the influence of the yeast on microalgal growth. Total initial cell number was 2×10^6 cells mL^{-1} for all treatments. The batch cultures were maintained at 26 °C with orbital shaking at 150 rpm under 100 μmol photons m^{-2} s^{-1} for 12 days. The pH of the medium was adjusted to 6.5 before autoclaving at 121 °C for 15 min.

Determination of cell growth and glucose in culture medium

Cell growth was determined by measuring cell counts and dry cell weight. The cell counts were by flow cytometry (FCM) (accuri C6, BD Biosciences). Cell dry weight was determined by centrifuging 2 mL cell of medium at 3800×*g* for 3 min, resuspending in distilled water three times, and then drying the residue to constant weight at 65 °C (Zhang et al. 2017b). The glucose concentration was analyzed with a biosensor (SBA-40D, Shandong Academy of Sciences, Jinan, Shandong, China).

Determination of pigments

Cells were collected and freeze-dried. Astaxanthin was determined by HPLC. The standards of astaxanthin, adonixanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll *a*, chlorophyll *b* were purchased from Sigma-Aldrich (USA). The pigments were extracted and measured with HPLC according to Chen et al. (2017). Briefly, 10 mg biomass was added with extraction solution (methanol/dichloromethane (3:1, *v*/*v*) containing 0.1% (*w*/*v*) butylated hydroxytoluene) and completely disrupted by a bead beater until the residue became colorless. Afterwards, the pigment dissolved in extraction solvent was dried under a continuous flow of nitrogen gas. Each sample was dissolved in 1 mL of methanol/MTBE (methyl tert-butyl ether) (1:1, *v*/*v*) and filtered through a 0.22-μm nylon filter for HPLC analysis. The process was conducted in darkness. The pigments were separated and determined by a HPLC (DIONEX P680, Thermo, Scientific, USA) equipped with a Water YMC Carotenoid C30 column (4.6 × 150 mm, 3 μm) and a 100 photodiode array detector. The peaks were scanned at 300–700 nm to analyze chlorophylls and carotenoids.

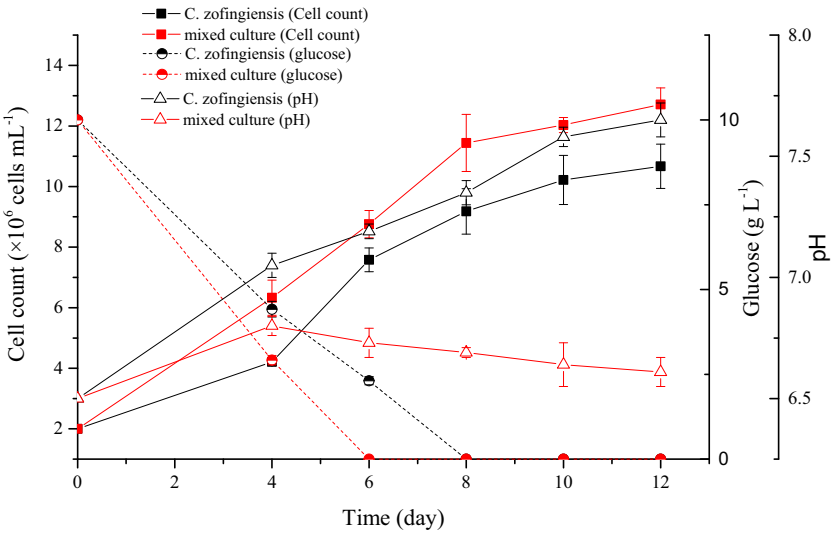

Fig. 1 Cell counts of *C. zofingiensis*, residual glucose concentration, and pH changes in mixed culture and monoculture. Mean ± SD ($n = 3$)

Lipid extraction and analysis

Total lipids were determined according to Bligh and Dyer (1959). A mixture of chloroform/methanol (2:1, *v*/*v*) was added to 10 mg biomass, and extracted for 1 h. Then, the mixture was centrifuged at 3800×*g* for 10 min to obtain a clear supernatant which was then transferred to a pre-weighed tube. Then, the supernatant was dried with a stream of N_2.

Fatty acid composition was determined by gas chromatography mass spectrometry (GC-MS) according to the method of Peng et al. (2015). Nonadecanoic acid was used as internal standard. Fatty acid methyl esters (FAMEs) were prepared by direct transesterification according to Lu et al. (2012). One milliliter of 5% KOH-CH_3OH and 100 mg nonadecanioc acid (C19:0) as internal standard were added to 10 mg of sample. The solution was incubated at 75 °C for 10 min and cooled. Then, 2 mL BF_3-CH_3OH was added and incubated at 75 °C for 10 min. A 0.5 mL saturated sodium solution and 2 mL hexane were added after cooling to room temperature and centrifuged at 5500 rpm for 10 min. The hexane layer was collected for fatty acid analysis. In order to visualize the intracellular lipid bodies, a confocal laser scanning microscope (CLSM) was used to analyze the stained cells as per Peng et al. (2015). Excitation wavelength was sent via a band-pass filter (460–490 nm) and emission light was used via a long-pass filter (510–540 nm). Laser transmission and scanning laser were stable in whole scans.

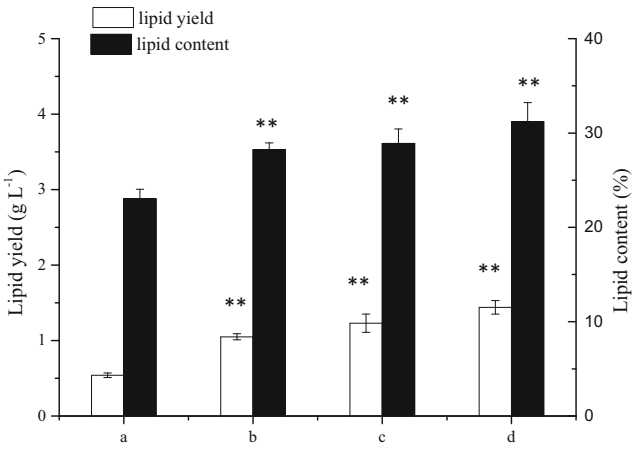

Fig. 2 The comparison of lipid content and total lipid in the co-culture and single culture. a, b, c, d Different seed ratios of microalgae to yeast (1:0, 1:1, 2:1, 3:1). Mean ± SD ($n = 3$). Statistically significant differences compared with *C. zofingiensis* in pure culture: $*p < 0.05$ and $**p < 0.01$

Statistical analysis

All experiments were performed in triplicate. Data are shown as mean value ± SD (standard deviation). Statistical analysis was performed using Origin 9.0 software. Statistical significances were evaluated by one-way ANOVA ($p < 0.05$).

Results

Lipid and astaxanthin accumulation

The biomass, lipid, and astaxanthin production from the co-culture of microalga *C .zofingiensis* and yeast *X. dendrorhous* were compared with pure culture of *C .zofingiensis*. As shown in Fig. 1, when the microalga and yeast were cultivated at the ratio of 1:1, in contrast with the pure culture of the microalga, the cell counts in the co-culture increased rapidly and the maximum cell counts of co-culture was 1.27×10^7 cells mL^{-1}. Moreover, the maximum dry weight in mixed culture was 3.71 ± 0.39 g L^{-1}, which was 1.58-fold that of the pure microalgal culture (2.35 ± 0.12 g L^{-1}) ($p < 0.05$). The glucose consumption rate in mixed culture was much faster than the monoculture of microalgae. No residual glucose was detected in the co-culture medium after 6 days. The

Table 1 Pigment profiles of *C. zofingiensis* in mixed culture and monoculture

Ratios (microalga to yeast)	Pigment content (mg g^{-1})						Astaxanthin yield (mg L^{-1})	Biomass (g L^{-1})
	Chlorophyll *a*	Chlorophyll *b*	Lutein	Zeaxanthin	Astaxanthin	Canthaxanthin		
1:0	1.52 ± 0.03	0.59 ± 0.01	0.85 ± 0.01	0.16 ± 0.01	1.08 ± 0.06	0.74 ± 0.01	2.54 ± 0.09	2.35 ± 0.12
1:1	1.68 ± 0.02*	0.47 ± 0.01*	0.77 ± 0.02	0.13 ± 0.01	1.11 ± 0.05	1.03 ± 0.02*	4.11 ± 0.27*	3.71 ± 0.39*
2:1	1.71 ± 0.02*	0.42 ± 0.01	0.65 ± 0.01	0.15 ± 0.01	1.14 ± 0.02	1.08 ± 0.02*	4.83 ± 0.31**	4.24 ± 0.35**
3:1	1.75 ± 0.01*	0.39 ± 0.01	0.69 ± 0.01	0.15 ± 0.01	1.19 ± 0.01*	1.13 ± 0.02*	5.5 ± 0.24**	4.62 ± 0.15**

Values are means ± SD ($n = 3$). Significant differentiation level with *$p < 0.05$ and **$p < 0.01$ by compared with the monoculture

pH of the mixed culture remained stable (approximately pH 6.60) during the cultivation. Conversely, the pH in the monoculture of the microalga increased sharply to pH 7.6.

As shown in Fig. 2 and Table 1, compared to the pure microalgal culture, enhanced biomass concentration, chlorophyll content (2.15 ± 0.03 mg g^{-1}), and canthaxanthin content (1.03 ± 0.02 mg g^{-1}) were obtained in the mixed-cultivation. Furthermore, both astaxanthin yield (4.11 ± 0.27 mg L^{-1}) and lipid content (28.23 ± 0.73%) in mixed culture were 1.61-fold and 1.23-fold higher than the microalgal culture. CLSM was employed to analyze the intracellular oil droplets. CSLM showed that the cells in the mixed culture were occupied by lipid bodies (Fig. 3). Compared to pure algae culture, more cell lipid droplets with stronger fluorescence were observed in mixed culture indicating that the lipid of both the microalga and the yeast in mixed culture is highly compared.

Increasing the amount of microalga to 2-fold and 3-fold in co-culture showed that the maximum biomass and lipid content was obtained in the mixed culture at the ratio of 3:1, measuring 4.62 ± 0.15 g L^{-1} and 31.2 ± 2.03%, respectively. Compared to the microalga monoculture, although biomass and lipid content were enhanced in co-culture, there was little increase in astaxanthin content when the amount of microalga was increased to 2-fold. However, both of astaxanthin content (1.19 ± 0.01 mg g^{-1}) and lipid content (31.2 ± 2.03%) could be improved simultaneously when the initial seed ratio of microalga to yeast was increased to 3:1. Furthermore, the contents of canthaxanthin (1.13 ± 0.02 mg g^{-1}) and chlorophyll *a* (1.75 ± 0.01 mg g^{-1}) in co-culture of microalga and yeast mixed at a ratio of 3:1 were also higher than that of the microalga in pure culture.

Fatty acid composition

Total fatty acid yields in all co-culture were significantly higher than that of monoculture of microalga and yeast (Table 2). Palmitic acid (17.05 ± 2.25%), oleic acid (50.25 ± 2.44%) and linolenic acid C18:2 (17.75 ± 2.74%) were the main fatty acids and together accounted for about 85.05% of fatty acids in the mixed culture at the 3:1 seed ratio of microalgae and yeast. The polyunsaturated fatty acids (PUFA) including (C18:2, C18:3, C16:2) in the 3:1 co-cultivation were 17.75 ± 2.74, 6.51 ± 1.05 and 3.32 ± 0.05%, respectively, which was much higher than that of the yeast monoculture. Furthermore, the total fatty acid content (31.2%) was enhanced by co-culture of microalga, and yeast at the ratio of 3:1 and the maximum total fatty acid yield (2.37 ± 0.39 g L^{-1}) in the 3:1 microalga and yeast culture was 2.72-fold higher than that of the alga monoculture.

Discussion

In this study, both the biomass concentration and lipid content in co-culture were higher than that of the microalga monoculture. It has been reported that mixed culture can promote the O_2/CO_2 gas exchange between microalgal and yeast cells and improve cell growth (Zhang et al. 2014). Moreover, the pH maintenance in co-culture may be beneficial to the growth of both of microalgae and yeast. The optimum pH of *X. dendrorhous* has been reported as 5.5–6.9 and organic acids synthesized during cultivation decrease the medium pH and hinder yeast growth (Johnson and Lewis 1979; Vaquez and

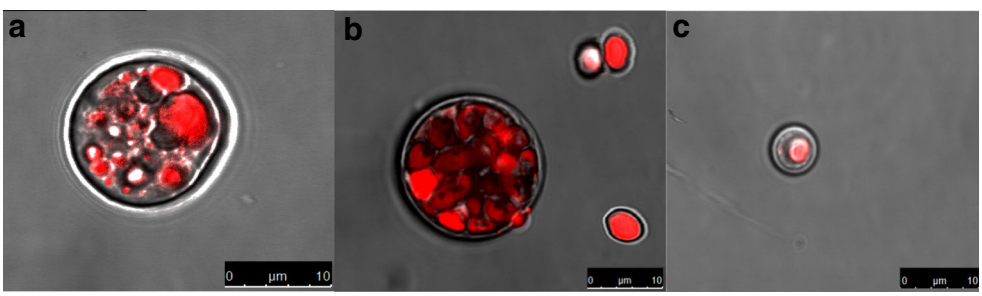

Fig. 3 Representative images captured by confocal laser scanning microscope (CLSM) of microalgae and yeast stained with BODIPY 505/515. **a** *C. zofingiensis* monoculture. **b** *C. zofingiensis* in mixed culture. **c** *X. dendrorhous* monoculture

Table 2 Fatty acid composition of *C. zofingiensis* cultivated in mixed culture and monoculture

	C16:0 (%)	C16:1 (%)	C16:2 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)	C 20:0(%)	Total fatty acid yield (g L^{-1})	Total fatty acid productivity (g L^{-1} day^{-1})
Mix 1	16.61 ± 1.31	1.57 ± 0.32	3.04 ± 0.62	3.24 ± 0.62	51.38 ± 4.22	17.90 ± 1.48	6.11 ± 0.65	0.16 ± 0.02	1.59 ± 0.19**	0.13 ± 0.01**
Mix 2	15.79 ± 0.94	1.54 ± 0.50	2.87 ± 0.43	3.33 ± 0.91	52.50 ± 2.98	17.38 ± 3.15	6.42 ± 0.94	0.18 ± 0.01	2.02 ± 0.08**	0.17 ± 0.06**
Mix 3	17.05 ± 2.25	1.88 ± 0.08	3.32 ± 0.05	3.02 ± 0.41	50.25 ± 2.44	17.75 ± 2.74	6.51 ± 1.05	0.20 ± 0.01	2.37 ± 0.39**	0.20 ± 0.01**
C. zofingiensis	17.43 ± 2.07	2.20 ± 0.53	3.40 ± 0.39	3.12 ± 0.71	48.95 ± 1.92	18.48 ± 1.09	6.15 ± 0.51	0.27 ± 0.04	0.87 ± 0.06	0.07 ± 0.01
P. rhodozyma	2.18 ± 0.44	–	–	6.33 ± 1.60	81.46 ± 5.25	8.59 ± 1.88	0.84 ± 0.05	0.60 ± 0.08	0.47 ± 0.07	0.04 ± 0.01

Values are means ± SD ($n = 3$). Significant differentiation level with $^{*}p < 0.05$ and $^{**}p < 0.01$ by compared with the monoculture

Martin 1998; Bhosale and Gadre 2001). The organic acids, such as acetate released by yeast cells, could be utilized by *C. zofingiensis* and thus reduce the inhibition of yeast growth by these metabolites (Xue et al. 2010; Liu et al. 2014). This may be a reason why the medium pH remains stable in mixed culture. In the algae monoculture the pH increased probably due to photosynthetic CO_2 uptake (Olguín et al. 2012; Borowitzka 2016).

Several studies have shown that *C. zofingiensis* accumulates lipids and astaxanthin under stress conditions such as high light and nitrogen starvation (Bar et al. 1995; Mulders et al. 2014). In this study, the mixed cultivation used glucose and urea in a molar C/N ratio of 180 as the sole carbon and nitrogen sources. It has been reported that a molar C/N ratio of 180 results in a higher astaxanthin content than a ratio of 30, urea is also regarded as better nitrogen for growth and lipid accumulation of microalgae compared with several cheap inorganic nitrogen sources (Cheirsilp et al. 2011; Liu et al. 2013). Due to the rapid growth of yeast in the early cultivation of the mixed culture, the nitrogen was consumed which then resulted in the lipid and astaxanthin accumulation. As the astaxanthin is located in lipid droplets, astaxanthin accumulation is associated with lipid synthesis (Mendoza et al. 1999; Zhekisheva et al. 2002; Solovchenko 2012). In the present study, lipid and astaxanthin accumulation were improved simultaneously in the mixed culture at a microalga to yeast ratio of 3:1, but not at other ratios. This suggests that some factors inhibited astaxanthin accumulation but had no effect on lipid accumulation. Zhekisheva et al. (2005) also found that inhibition of lipid accumulation inhibited astaxanthin biosynthesis; however, inhibiting astaxanthin accumulation had no effect on lipid accumulation.

The fatty acid composition is important in evaluating the qualities of biodiesel (Knothe 2013). The main fatty acids of the monocultures of *C. zofingiensis* and X. *dendrorhous* were linoleic acid, oleic acid, and palmitic acid (Sanderson and Jolly 1994; Zhang et al. 2016). Linoleic acid, oleic acid, and palmitic acid were also the main fatty acid of the co-cultures with the main fatty acids similar to plant lipid (Li et al. 2007). This indicates that the lipid in the present study could be utilized as a feedstock for biodiesel production. Microalgal fatty acid composition is influenced by culture conditions (Guschina and Harwood 2013). Therefore, approaches such as fed-batch, continuous culture in this mixed-cultivation will be carried out to improve the lipid quality.

In conclusion, compared to monoculture of *C. zofingiensis*, higher biomass and astaxanthin production were obtained in co-culture of *C. zofingiensis* and *X. dendrorhous*. Enhanced lipid and astaxanthin content was achieved simultaneously with the increasing amount of microalga in co-culture at the ratio of 3:1 (microalgae to yeast). Moreover, the mixed system affected culture pH in a way which benefits the growth of both species. This study has provided a new strategy to enhance

simultaneously astaxanthin and lipid production in co-culture under mixotrophic cultivation.

Funding information This work was funded by the Science and Technology Program of Guangdong (Grant nos. 2016A010105001 and 2015A20216003), the Sciences and Technology of Guangzhou (Grant no. 201704030084), the Science and Technology Program in Marine and Fishery of Guangdong (Grant no. A201401C01).

References

Ambati RR, Phang SM, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications-a review. Mar Drugs 12:128–152

Bar E, Rise M, Vishkautsan M, Arad S (1995) Pigment and structural changes in *Chlorella zofingiensis* upon light and nitrogen stress. J Plant Physiol 146(4):527–534

Bhosale P, Gadre RV (2001) β-Carotene production in sugarcane molasses by a *Rhodotorula glutinis* mutant. J Ind Microbiol Biotechnol 26:327–332

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Physiol Pharmacol 37:911–917

Borowitzka MA (2016) Algal physiology and large-scale outdoor cultures of microalgae. In: Borowitzka MA, Beardall J, Raven JA (eds) The physiology of microalgae. Springer, Dordrecht, pp 601–652

Borowitzka MA, Huisman JM, Osborn A (1991) Culture of the astaxanthin-producing green alga *Haematococcus pluvialis* 1. Effects of nutrients on growth and cell type. J Appl Phycol 3:295–304

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Plant 108:111–117

Cai S, Hu C, Du S (2007) Comparisons of growth and biochemical composition between mixed culture of alga and yeast and monocultures. J Biosci Bioeng 104:391–397

Cheirsilp B, Kitcha S, Torpee S (2011) Co-culture of an oleaginous yeast *Rhodotorula glutinis* and a microalga *Chlorella vulgaris* for biomass and lipid production using pure and crude glycerol as a sole carbon source. Ann Microbiol 62:987–993

Chen J, Wei D, Pohnert G (2017) Rapid estimation of astaxanthin and the carotenoid-to-chlorophyll ratio in the green microalga *Chromochloris zofingiensis* using flow cytometry. Mar Drugs 15(7):E231

Dominguez-Bocanegra A, Ponce-Noyola T, Torres-Munoz J (2007) Astaxanthin production by *Phaffia rhodozyma* and *Haematococcus pluvialis*: a comparative study. Appl Microbiol Biotechnol 75:783–791

Dong Q-L, Zhao X-M (2004) In situ carbon dioxide fixation in the process of natural astaxanthin production by a mixed culture of *Haematococcus pluvialis* and *Phaffia rhodozyma*. Catal Today 98: 537–544

Guschina IA, Harwood JL (2013) Algal lipids and their metabolism. In: Borowitzka MA, Moheimani NR (eds) Algae for biofuels and energy. Springer, Dordrecht, pp 17–36

Ip P-F, Wong K-H, Chen F (2004) Enhanced production of astaxanthin by the green microalga *Chlorella zofingiensis* in mixotrophic culture. Process Biochem 39:1761–1766

Johnson EA, Lewis MJ (1979) Astaxanthin formation by the yeast *Phaffia rhodozyma*. J Gen Microbiol 115:173–183

Kim D-Y, Vijayan D, Praveenkumar R, Han J-I, Lee K, Park J-Y, Chang W-S, Lee J-S, Oh Y-K (2016) Cell-wall disruption and lipid/astaxanthin extraction from microalgae: *Chlorella* and *Haematococcus*. Bioresour Technol 199:300–310

Knothe G (2013) Production and properties of biodiesel from algal oils. In: Borowitzka MA, Moheimani NR (eds) Algae for biofuels and energy. Springer, Dordrecht, pp 207–221

Li Y, Zhao Z, Bai F (2007) High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. Enzym Microb Technol 41:312–317

Liu J, Sun Z, Gerken H, Liu Z, Jiang Y, Chen F (2014) *Chlorella zofingiensis* as an alternative microalgal producer of astaxanthin: biology and industrial potential. Mar Drugs 12:3487–3515

Liu JS, Z.; Zhong, Y., Gerken H, Huang J, Chen F (2013) Utilization of cane molasses towards cost-saving astaxanthin production by a *Chlorella zofingiensis* mutant. J Appl Phycol 25:1447–1456

Liu L, Chen J, Lim P-E, Wei D (2018) Dual-species cultivation of microalgae and yeast for enhanced biomass and microbial lipid production. J Appl Phycol. https://doi.org/10.1007/s10811-018-1526-y

Lu N, Wei D, Jiang X-L, Chen F, Yang S-T (2012) *Fatty acids profiling and biomarker identification in snow alga Chlamydomonas nivalis* by NaCl stress using GC/MS and multivariate statistical analysis. Anal Lett 45:1172–1183

Mendoza H, Martel A, Jiménez del Río M, García Reina G (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11:15–19

Mulders KJM, Janssen JH, Martens DE, Wijffels RH, Lamers PP (2014) Effect of biomass concentration on secondary carotenoids and triacylglycerol (TAG) accumulation in nitrogen-depleted *Chlorella zofingiensis*. Algal Res 6, Part A:8–16

Nichols HW, Bold HC (1965) *Trichosarcina polymorpha* gen. et. sp. nov. J Phycol 1:34–38

Olguín EJ, Olguín EJ, Giuliano G, Porro D, Tuberosa R, Salamini F (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. Biotechnol Adv 30:1031–1046

Osterlie M, Bjerkeng B, Liaaen-Jensen S (1999) Accumulation of astaxanthin all-*E*, 9*Z* and 13*Z* geometrical isomers and 3 and 3' *RS* optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. J Nutr 129:391–398

Peng H, Wei D, Chen F, Chen G (2015) Regulation of carbon metabolic fluxes in response to CO_2 supplementation in phototrophic *Chlorella vulgaris*: a cytomic and biochemical study. J Appl Phycol 28:737–745

Qin L, Liu L, Wang Z, Chen W, Wei D (2018) Efficient resource recycling from liquid digestate by microalgae-yeast mixed culture and the assessment of key gene transcription related to nitrogen assimilation in microalgae. Bioresour Technol 264:90–97

Sanderson GW, Jolly SO (1994) The value of *Phaffia* yeast as a feed ingredient for salmonid fish. Aquaculture 124:193–200

Santos CA, Reis A (2014) Microalgal symbiosis in biotechnology. Appl Microbiol Biotechnol 98:5839–5846

Solovchenko AE (2012) Physiological role of neutral lipid accumulation in eukaryotic microalgae under stress. Russ J Plant Physiol 59:167–176

Sun N, Wang Y, Li Y-T, Huang J-C, Chen F (2008) Sugar-based growth, astaxanthin accumulation and carotenogenic transcription of heterotrophic *Chlorella zofingiensis* (Chlorophyta). Process Biochem 43: 1288–1292

Vaquez M, Martin AM (1998) Optimization of *Phaffia rhodozyma* continuous culture through response surface methodology. Biotechnol Bioeng 57:314–320

Xue F, Miao J, Zhang X, Tan T (2010) A new strategy for lipid production by mix cultivation of *Spirulina platensis* and *Rhodotorula glutinis*. Appl Biochem Biotechnol 160:498–503

Yen HW, Chen PW, Chen LJ (2015) The synergistic effects for the co-cultivation of oleaginous yeast-*Rhodotorula glutinis* and microalgae-*Scenedesmus obliquus* on the biomass and total lipids accumulation. Bioresour Technol 184:148–152

Zhang K, Zheng J, Xue D, Ren D, Lu J (2017a) Effect of photoautotrophic and heteroautotrophic conditions on growth and lipid production in *Chlorella vulgaris* cultured in industrial wastewater with the yeast *Rhodotorula glutinis*. J Appl Phycol 29:2783–2788

Zhang Z, Huang JJ, Sun D, Lee Y, Chen F (2017b) Two-step cultivation for production of astaxanthin in *Chlorella zofingiensis* using a patented energy-free rotating floating photobioreactor (RFP). Bioresour Technol 224:515–522

Zhang Z, Ji H, Gong G, Zhang X, Tan T (2014) Synergistic effects of oleaginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* for enhancement of biomass and lipid yields. Bioresour Technol 164:93–99

Zhang Z, Sun D, Mao X, Liu J, Chen F (2016) The crosstalk between astaxanthin, fatty acids and reactive oxygen species in heterotrophic *Chlorella zofingiensis*. Algal Res 19:178–183

Zhekisheva M, Boussiba S, Khozina-Goldberg I, Zarka A, Cohen Z (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 38:325–331

Zhekisheva M, Zarka A, Khozin-Goldberg I, Cohen Z, Boussiba S (2005) Inhibition of astaxanthin synthesis under high irradiance does not abolish triacylglycerol accumulation in the green alga *Haematococcus pluvialis* (Chlorophyceae). J Phycol 41:819–826