

# Utilization of High-Fructose Corn Syrup for Biomass Production Containing High Levels of Docosahexaenoic Acid by a Newly Isolated *Aurantiochytrium* sp. YLH70

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**Abstract** High-fructose corn syrup (HFCS) is an agro-source product and has been the most commonly used substitute for sugar as sweetener in food industry due to its low price and high solution property. In this study, the F55 HFCS, rich in fructose and glucose, was first tested for biomass and docosahexaenoic acid productions as a mixed carbon source by a newly isolated *Aurantiochytrium* sp. YLH70. After the compositions of the HFCS media were optimized, the results showed that the HFCS with additions of metal ion and vitamin at low concentrations was suitable for biomass and docosahexaenoic acid productions and the metal ion and sea salt had the most significant effects on biomass production. During the 5-l fed-batch fermentation, total HFCS containing 180 g l<sup>-1</sup> reducing sugar was consumed and yields of biomass, lipid, and DHA could reach 78.5, 51, and 20.1 g l<sup>-1</sup>, respectively, at 114 h. Meanwhile, the daily productivity and the reducing sugar conversion yield for docosahexaenoic acid were up to 4.23 g l<sup>-1</sup> day<sup>-1</sup> and 0.11 g g<sup>-1</sup>. The fatty acid profile of *Aurantiochytrium* sp. YLH70 showed that 46.4 % of total fatty acid was docosahexaenoic acid, suggesting that *Aurantiochytrium* sp. YLH70 was a promising DHA producer.

**Keywords** *Aurantiochytrium* sp. YLH70 · Docosahexaenoic acid (DHA) · Fermentation · High-fructose corn syrup (HFCS) · Response surface methodology (RSM)

## Introduction

High-fructose corn syrup (HFCS), a corn-made product that mainly contains fructose and glucose, has been the traditional and most commonly used substitute for sugar as sweetener in

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food and beverage industry due to its low price and sweetness comparable with the sucrose [1, 2]. Moreover, since HFCS contains large amount of fermentable sugar, nice flavor, and high solution properties, it was used in microbial fermentations for productions of yoghurt [3] and proteinase [4]. However, utilization of the HFCS for microbial lipids rich in docosahexaenoic acid (DHA) has not been investigated.

DHA is a member of  $\omega$ -3 polyunsaturated fatty acids (PUFAs) and has been recently reported to have beneficial effects on preventing cardiovascular diseases, cancer, schizophrenia, and Alzheimer's disease. DHA also plays a significant role in infant nervous development and is an essential nutrient for cultured animals in feed industry [5]. *Aurantiochytrium* sp. is a heterotrophic marine thraustochytrids microorganism which can grow on various carbon sources. It has been attracted much attentions for its high growth rate and capabilities to produce a large amount of DHA when grown on glucose and fructose [6]. However, compared with the fish oil DHA, microbial DHA has high production cost [7]. For decreasing production cost of microbial DHA, higher space-time productivities must be obtained by culture optimization, high-cell-density cultivation, and utilization of economic materials [8, 9]. The growth rate, biomass, and DHA content were considered as key factors for the space-time productivity [10]. Many inexpensive materials, such as cane molasses [11], waste glycerol [12], sweet sorghum juice [13], and shochu distillery wastewater [14], have been used for DHA productions by thraustochytrids. However, utilization of the HFCS for DHA production by *Aurantiochytrium* sp. has never been investigated.

The response surface methodology (RSM) was considered to be a more effective mathematical and statistical technique for optimizing conditions. By providing statistical model and experiment, RSM reveals interactions among the relative factors and calculates the optimal condition [15]. For example, after optimizations of glucose, yeast extract, NaCl, pH, and incubation time by RSM, the DHA production by *Schizochytrium* sp. S31 reached  $516 \text{ mg l}^{-1}$  [16]. The amount of eicosapentaenoic acid (EPA) for the marine bacteria 717 increased from 9 to  $45 \text{ mg g}^{-1}$  biomass after RSM performances, showing the significant effects of temperature, pH, and dissolved oxygen on the EPA production [17].

In this study, we report our findings on the utilization of HFCS by a newly isolated *Aurantiochytrium* sp. YLH70 for biomass and DHA productions. The strain isolation, identification, medium optimization, and fermentation performance are also presented here.

## Materials and Methods

### Materials

The F55 high-fructose corn syrup (HFCS) was purchased from the Deanfu Sugar Ltd. (Xiaogan, Hubei, China), and its main compositions are shown in Table 1. The glucose, fructose, xylose, maltose, sucrose, glycerol, starch, lactose, olive oil, and corn steep liquor were obtained from the Aladdin Industrial Inc. (Shanghai, China). The yeast extract and peptone were obtained from the OXOID Scientific Inc. (England). The bean cake powder was obtained from the Guanqian Industrial Inc. (Tianjin, China). The nonadecanoic acid methyl ester and the docosahexaenoic acid methyl ester standards were purchased from Sigma-Aldrich (St. Louis, USA). The BF3-methanol was purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China).

**Table 1** Compositions of the F55 high-fructose corn syrup (HFCS)

Component	Fructose	Glucose	Total reducing sugar	Solids	Undissolved substance
Concentration (g l <sup>-1</sup> )	419 ± 7.2	321 ± 6.5	800 ± 5.62	77.5 ± 1.5	0.0038 ± 0.06

Data for the total reducing sugar are given as mean ± SD,  $n = 3$

## Samplings and Isolations of *Aurantiochytrium* Strains

Leaves from *Kandelia* plants were collected from the mangrove ecosystem in Yueqing Bay, Zhejiang province, China. Strains of *Aurantiochytrium*-like microorganisms were obtained from the samples using the pollen baiting technology reported by Gupta et al. [18]. The purified *Aurantiochytrium*-like colonies were preserved in glycerol tubes and at -80 °C for further study.

## Strain and Cultivations

Strain YLH 70 was cultivated on the GPY medium (60 g l<sup>-1</sup> glucose, 20 g l<sup>-1</sup> yeast extract, 20 g l<sup>-1</sup> sea salt) in flasks at 28 °C and 180 rpm in a horizontal rotator. For optimizations, as described as below, the GPY medium was a base medium and one factor in the medium was changed while the others were fixed.

## Measurement of Biomass

The biomass was measured as the dry cell weight (DCW). Fifty milliliters of cell cultures were centrifuged at 10,000 rpm and 4 °C for 10 min, and the cell pellets were then washed twice with distilled water. The washed cell pellets were then lyophilized to constant weight at 40 °C for 48 h and weighted.

## Measurement of Reducing Sugar

Reducing sugar in the fermented media or HFCS was measured by the Nelson-Somogyi method, and the amount of reducing sugar was calculated [19]. Glucose and fructose were separated and analyzed by Waters Alliance 2414 HPLC system with Waters Refractive Index Detector. Hypersil NH<sub>2</sub> column (4.6 mm \* 250 mm, Yilite Ltd., China) was used with an acetonitrile/water (80:20) mobile phase for isocratic elution. The flow rate was 1.4 ml/min and injection volume was 10 µl. The column temperature was kept at 30 °C. Glucose and fructose, which were used as standard for quantitative analysis, were obtained from Sigma-Aldrich (St. Louis, USA).

## Lipid Extraction and Analysis of Fatty Acid Composition

The lipid extraction and analysis of fatty acid composition was performed based on the method reported by Gao et al. with moderate modifications [20]. The lyophilized cell was pestled into fine powder with mortar under liquid nitrogen. The cell powder was extracted into 100 ml chloroform/methanol (2:1, v/v) at room temperature. The lipid extract was dried by evaporation and weighed. For fatty acid analysis, 0.1 g of cell powder was mixed with 5 ml of 0.4 M

methanolic KOH and incubated at 60 °C for 1 h, followed by an esterification procedure in 5 ml of BF<sub>3</sub>-methanol (14 %, w/w) reagent at 60 °C for another 1 h. The fatty acid methyl esters (FAMES) was extracted with 5 ml of hexane and analyzed by Agilent 6890N GC using an HP-INNOWAX column (30 m \* 0.25 mm, 0.25 µm film thickness, Agilent Technologies) with the He as carrier gas. The temperature-controlling procedure was as follows: from 100 to 240 °C at 15 °C min<sup>-1</sup> and maintained at 240 °C for another 10 min; the temperature of the injection port and flame ionization port was 250 °C. The nonadecanoic acid methyl ester was used as the internal standard.

### Molecular Identification of the Strain YLH70 by 18S rDNA Sequencing

The genomic DNA of the strain YLH70 was extracted by a universal genomic DNA extraction kit (Takara, Japan) according to the kit protocol. The 18S rDNA was amplified with genomic DNA as a template using the forward primer 16S1N (5'-TCCTGCCAGTAGTCATATGC-3') and the reverse primer 16S2N (5'-TGATCCTCYGCAGGTTAC-3') [21]. The 50-µl polymerase chain reaction (PCR) system contained 5 µl ×10 PCR buffer, 10 mM dNTP, 10 µM each primer, 1.25 U Taq DNA polymerase (Takara, Japan), and 1 µg genomic DNA. The PCR program was run for 5 min at 94 °C, followed by 35 cycles each consisted of 30 s at 94 °C, 30 s at 50 °C, and 2 min at 72 °C. The final extension consisted of 5 min at 72 °C. The PCR product was purified from gel electrophoresis using the Axygen DNA extraction kit (Axygen, Hangzhou, China) and cloned into the pMD-18T (Takara, Japan). The final vectors were sent to the Sangon Biotech Ltd. (Shanghai, China) for DNA sequencings.

The resulting 18S rDNA sequences were aligned to the nucleotide sequences in the database of the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) for identification of the isolated strain. The 18S rDNA sequences of related microorganisms were downloaded and analyzed by MEGA software (Version 4.0) to construct a neighbor-joining (NJ) tree with bootstrap 1000 replicates.

### Optimizations of Media Compositions for DCW, Lipid, and DHA Productions

“One-at-a-time” method was used to select suitable carbon and nitrogen sources of the GPY medium for DCW, lipid, and DHA productions by *Aurantiochytrium* sp. YLH70. Optimizations were performed at 50-ml flash level. Strain was cultivated at 28 °C and 180 rpm for 5 days. Ten carbon sources, including glucose, fructose, xylose, maltose, sucrose, glycerol, starch, lactose, olive oil, and HFCS, and four nitrogen sources, including yeast extract, peptone, corn steep liquor, and bean cake powder, were tested, while the other factors of media were fixed as the initial condition. The effects of different concentrations of HFCS (40, 60, 80, 100, 120, 140 ml l<sup>-1</sup>) and yeast extract (5, 10, 15, 20, 30 g l<sup>-1</sup>) on the DCW, lipid, and DHA productions were also studied.

As shown in Table 2, a central composite design (CCD) for five variables, which represent compositions of the medium at five coded levels (+2, +1, 0, -1, -2) was used for experimental design and analysis by response surface methodology (RSM). Among these compositions, the HFCS, yeast extract, sea salt, metal ion, and vitamin were tested for their significances and the optimal levels for production of DCW by *Aurantiochytrium* sp. YLH70. The CCD and analysis of variance (ANOVA) were performed with the Design Expert software package (Version 7.0, State-Ease Inc., Minneapolis, MN, USA).

**Table 2** Variables and their levels used for the central composite design (CCD) experiment

Variables	Units	Code	Level				
			-2	-1	0	1	2
HFCS	ml l <sup>-1</sup>	A	80	90	100	110	120
Yeast extract	g l <sup>-1</sup>	B	10	15	20	25	30
Sea salt	g l <sup>-1</sup>	C	10	15	20	25	30
Metal ion	l <sup>a</sup>	D	0	0.5	1	1.5	2
Vitamin	l <sup>b</sup>	E	0	0.5	1	1.5	2

<sup>a</sup> One unit of metal ion was composed of 5 mg l<sup>-1</sup> FeSO<sub>4</sub>·7H<sub>2</sub>O, 1 mg l<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O, 1 mg l<sup>-1</sup> ZnSO<sub>4</sub>·7H<sub>2</sub>O, and 0.5 mg l<sup>-1</sup> MnSO<sub>4</sub>·H<sub>2</sub>O

<sup>b</sup> One unit of vitamin B was composed of 0.05 mg l<sup>-1</sup> thiamine and 0.005 mg l<sup>-1</sup> biotin

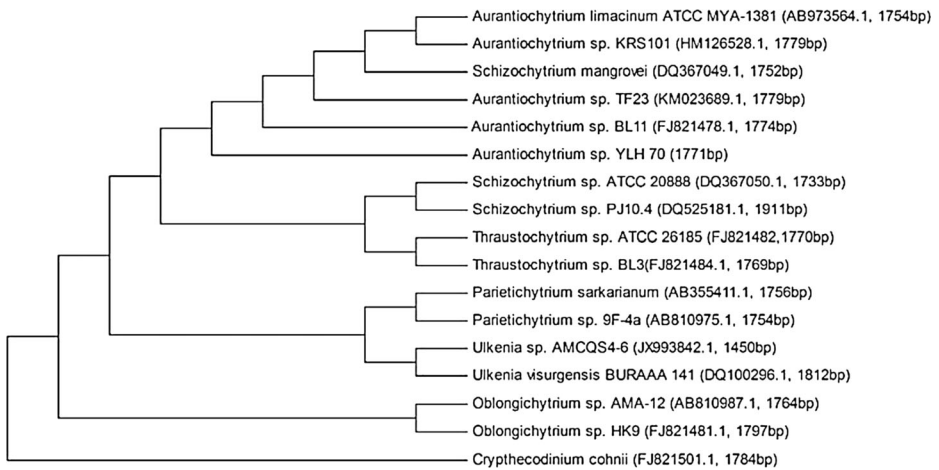
## Bioreactor Cultivations

A RALF 5 l bioreactor (Bioengineering, Swiss) was used to perform the fed-batch bioreactor cultivation using the medium for *Aurantiochytrium* sp. YLH70 DCW production optimized by CCD. The preculture was prepared in a 500-ml flask and then transferred into the 5 l bioreactor containing 4 l medium. One milliliter of antifoam chemical (1 g l<sup>-1</sup>, Sigma) was used to suppress foaming. The fermentation was set at 25 °C with aeration and agitation rate kept at 1 vvm and 400 rpm, respectively. During the fermentation, when the concentration of reducing sugar in medium dropped to 10 g l<sup>-1</sup>, the sterilized HFCS was supplied for increasing up to 60 g l<sup>-1</sup> reducing sugar in medium. Fifty milliliters of sample was taken at 6-h intervals over the entire cultivation (120 h) for DCW, reducing sugar, lipid, and fatty acid composition analyses.

## Results and Discussion

### Isolation and Identification of Strain YLH70

About 100 purified strains of *Aurantiochytrium*-like microorganisms were isolated from the fallen leaf samples. The DCW and DHA contents of these strains ranged from 7.66 to 14.5 and 0.18 g l<sup>-1</sup> to 1.98 g l<sup>-1</sup>, respectively. Based on the DCW and DHA yields, the strain YLH70 was selected for further study. Using microscope inspection (data not shown) and molecular phylogeny reconstruction, the strain YLH70 was confirmed to be an *Aurantiochytrium* strain. In the neighbor-joining (NJ) tree of 18S rDNA (Fig. 1), the strain YLH70 was located in the well-supported monophyletic group with the strains of *Aurantiochytrium* sp. BL11, KRS101, and TF23. Moreover, as shown in Table 3, the fatty acid profile of the YLH70 strain is simple, with palmitic acid (C16:0, 33.4 % TFA), eicosapentaenoic acid (C22:5, 9.71 % TFA), and DHA (C22:6, 46.3 % TFA) as main fatty acids, which was used as a tool for thraustochytriaceae strain identification and very similar to those for other *Aurantiochytrium* sp. Strains [22]. Thus, the strain YLH70 was designated as *Aurantiochytrium* sp. YLH70.



**Fig. 1** Neighbor-joining tree based on 18S rDNA sequence data. The numbers at each internal branch show the bootstrap values (%) for the nodes calculated by 1000 replicates

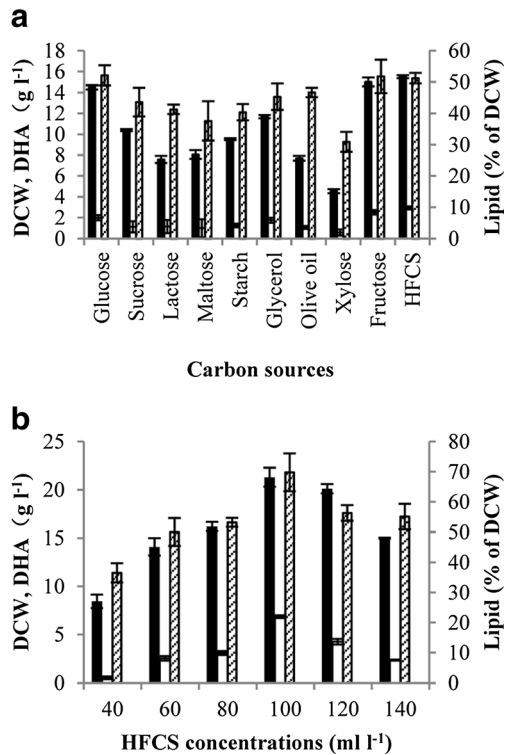
## Optimizations of Media Components

Various carbon sources were used as substrates to investigate the DCW and DHA yields of *Aurantiocytrium* sp. YLH70 (Fig. 2a). Among the single carbon sources, fructose was the optimal carbon source with  $15 \text{ g l}^{-1}$  DCW,  $7.77 \text{ g l}^{-1}$  lipid, and  $2.5 \text{ g l}^{-1}$  DHA followed by glucose with  $14.5 \text{ g l}^{-1}$  DCW,  $7.55 \text{ g l}^{-1}$  lipid, and  $1.98 \text{ g l}^{-1}$  DHA. These results were similar to those for *Schizocytrium limacinum* SR21 [6]. Liang et al. reported that mixed carbon source, such as sweet sorghum juice (mainly composed of glucose, fructose, and sucrose), resulted in higher DHA yield than that from single glucose at the same concentration of sugar [13]. Thus, the mixed carbon source, F55 HFCS, was also tested for DCW, lipid, and DHA productions by *Aurantiocytrium* sp. YLH70. The composition of the F55 HFCS is shown in Table 1, showing an up to  $800 \text{ g l}^{-1}$  of reducing sugar with fructose and glucose as main sugars. As shown in Fig. 2a, HFCS was a superior carbon source to fructose and glucose for DHA production, which was similar to the result from Liang et al. [13]. The explanation for

**Table 3** Fatty acid profiles of *Aurantiocytrium* sp. YLH70

Fatty acids	Content (% TFA)
C14:0	3.16
C15:0	3.87
C16:0	33.4
C16:1 (n-7)	0.78
C17:0	0.27
C18:0	1.09
C20:0	0.25
C20:4 (n-6, ARA)	0.55
C20:5 (n-3, EPA)	0.65
C22:5 (n-6, EPA)	9.71
C22:6 (n-3, DHA)	46.3

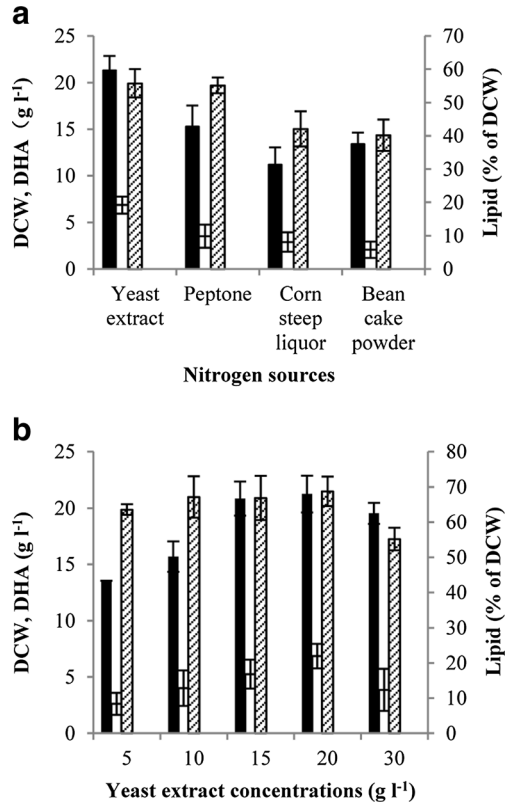
**Fig. 2** Effects of various carbon sources (a) and high fructose corn syrup (HFCS) concentrations (b) on dry cell weight (DCW), docosahexaenoic acid (DHA) yields, and lipid productions for *Aurantiochytrium* sp. YLH70. All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean. DCW, close bar; DHA yield, open bar; lipid production, slash bar



these results may be as follows: (1) some trace substrates in HFCS at some concentration could stimulate cell growth and DHA production and (2) less amount of glucose in HFCS than pure glucose could decrease the inhibition effect of glucose on the cell growth and DHA production. HFCS, a material which originated from plant starch, is cheaper than fructose and sucrose and has almost the same price as that of glucose. As shown in Fig. 2b, the optimal concentration of HFCS for DCW, lipid, and DHA yields was 100 ml l<sup>-1</sup>, showing that *Aurantiochytrium* sp. YLH70 could endure up to 96 g l<sup>-1</sup> reducing sugar (120 ml l<sup>-1</sup> HFCS) in media, which was as same as *S. limacinum* SR21 [6] and even higher than *Schizochytrium mangrovei* PQ6 [23] and *Aurantiochytrium* sp. KRS101 [24]. A further increase in concentration of carbon source inhibited cell growth and DHA production, while had little effect on lipid production. As shown in Fig. 3a, yeast extract was the optimal nitrogen source for DCW and DHA yields by *Aurantiochytrium* sp. YLH70, followed by peptone, corn steep liquor, and bean cake powder. Moreover, the optimal DCW, lipid, and DHA values of 21.3, 14.6, and 6.86 g l<sup>-1</sup> were obtained at 20 g l<sup>-1</sup> yeast extract (Fig. 3b). The carbon/nitrogen ratio in medium plays a significant role in lipid and DHA productions, and high C/N ratio was effective for accumulations of lipid and DHA. Under nitrogen depletion condition, residual carbon source in medium flow to the lipid synthesis by producing acetoacetyl coenzyme A, which was the structural unit for lipid and DHA synthesis [25]. As shown in Fig. 2b and Fig. 3b, it was deduced that when C/N ratio in medium was 5 (100 g l<sup>-1</sup> reducing sugar and 20 g l<sup>-1</sup> yeast extract), the optimal lipid and DHA yields were obtained, with the same tendency as in *S. mangrovei* PQ6 [23] and *Aurantiochytrium* sp. SD116 [20]. Meanwhile,



**Fig. 3** Effects of various nitrogen sources (a) and yeast extract concentrations (b) on DCW, DHA yields, and lipid productions for *Aurantiochytrium* sp. YLH70. All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean. DCW, close bar; DHA yield, open bar; lipid production, slash bar



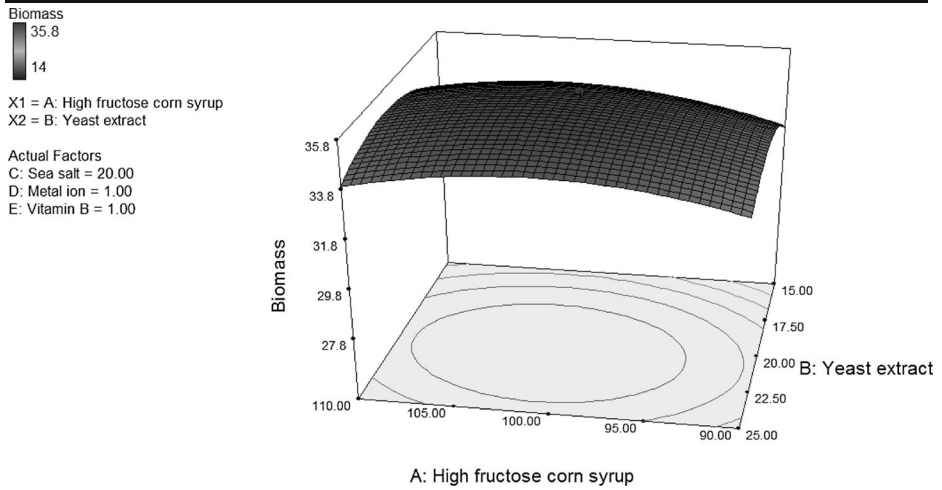
the C/N ratio had almost the same effect on the lipid and DHA accumulations, showing the lipid and DHA synthesis are related biological processes.

In addition to the macronutrients (carbon and nitrogen sources), micronutrients (vitamin and trace element) and sea salt are both required for growth and DHA productions by *Aurantiochytrium* sp. [26, 27]. Thus, the HFCS, yeast extract, sea salt, two kinds of vitamins (thiamine and biotin) and four kinds of metal ions ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ) were optimized using central composite design (CCD) and the interactive effects of these factors were also analyzed. A summary of variables and their variations was listed in Table 2. The regression model of the CCD results was fit to a second-order polynomial:

$$\begin{aligned}
 Y = & +35.47 + 0.28A + 0.53B + 0.075C + 1.63D + 0.24E - 0.16AB - 0.082AC - 0.043AD \\
 & + 0.12AE + 0.18BC + 0.17BD - 0.069BE \\
 & + 0.14CD - 0.019CE - 0.13DE - 0.89A^2 - 1.21B^2 - 5.04C^2 - 2.66D^2 - 1.01E^2.
 \end{aligned}$$

where  $Y$ ,  $A$ ,  $B$ ,  $C$ ,  $D$ , and  $E$  are the DCW, HFCS, yeast extract, sea salt, metal ion, and vitamin, respectively. The plot described by model  $Y$  is shown in Fig. 4, showing that the maximum DCW value of  $35.8 \text{ g l}^{-1}$  was obtained when the optimal concentrations for  $A$ ,  $B$ ,  $C$ ,  $D$ , and  $E$  were  $100 \text{ ml l}^{-1}$ ,  $20 \text{ g l}^{-1}$ ,  $20 \text{ g l}^{-1}$ ,  $1.0$ , and  $1.0$ , respectively, and the model predicted the optimal DCW value of  $35.5 \text{ g l}^{-1}$ . As shown in Table 4, the fit of the model  $Y$  was evaluated by the coefficient of determination,  $R^2$ , which was 0.966, indicating that the model





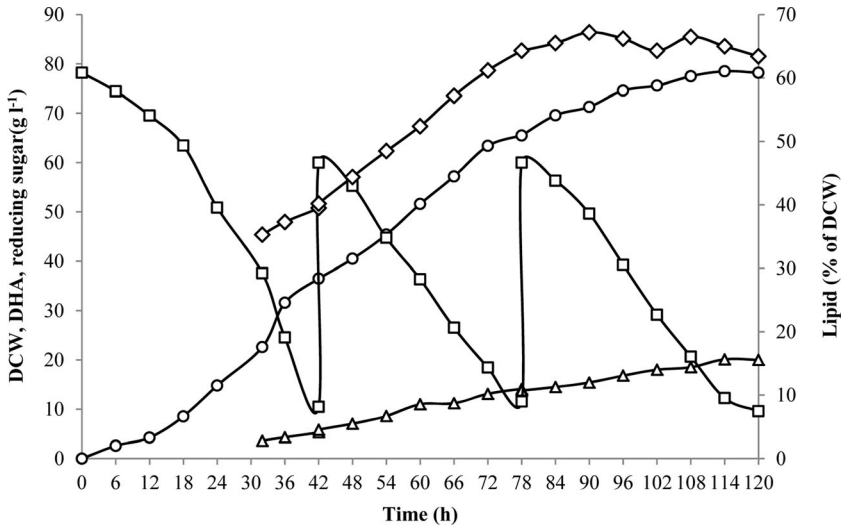
**Fig. 4** 3-D surface plot for DCW in *Aurantiochytrium* sp. YLH70

could explain 96.6 % of the variability. The model  $F$  value of 40.6 implies the model is significant. There is only a 0.01 % chance that a “model  $F$  value” this large could occur due to noise.  $P$  value less than 0.0001 and sum of squares more than 100 indicate model terms are significant; thus, Table 4 shows that the metal ion ( $D$ ) and the squares of sea salt and metal ion ( $C^2$  and  $D^2$ ) had significant effects on the DCW. Through Plackett-Burman design, Chi et al. also found that trace metals significantly affected growth and DHA yield of *Aurantiochytrium limacinum* SR21 [28]. It is well known that trace metals, especially manganese, iron, cobalt, nickel, copper, zinc, and molybdenum are essential micronutrients for growths of various species of thraustochytrids [27]. Therefore, metal ions were usually used in fermentations of *Aurantiochytrium* [29, 30]. *Aurantiochytrium* sp. YLH70 was able to endure a range of 10 to

**Table 4** Analysis of variance (ANOVA) of the CCD experiment and significance of the coefficients of regression

Source	Sum of squares	$df$	Mean square	$F$ value	$P$ value
Model	1273.338278	20	63.66691388	40.596155	<0.0001
B-Yeast extract	11.04601	1	11.04601	7.0433056	0.0128*
D-Metal ion	106.86361	1	106.86361	68.139814	<0.0001**
$A^2$	25.2689405	1	25.2689405	16.112322	0.0004*
$B^2$	47.1323405	1	47.1323405	30.053158	<0.0001**
$C^2$	812.4077405	1	812.4077405	518.01836	<0.0001**
$D^2$	227.0367405	1	227.0367405	144.76622	<0.0001**
$E^2$	32.8779405	1	32.8779405	20.964075	<0.0001**
$R^2 = 0.966$			Adeq Precision = 25		

\*\* $P < 0.0001$  indicates that model terms are highly significant at the 0.01 % level; \* $P < 0.05$  indicates that model terms are significant at the 5 % level



**Fig. 5** Fed-batch fermentation of *Aurantiochytrium* sp. YLH70. Reducing sugar, open squares; DCW, open circles; DHA yield, open triangles; lipid production, open diamonds

30 g l<sup>-1</sup> sea salt, and the optimal one for DCW production was 20 g l<sup>-1</sup> sea salt. It had been reported that *A. limacinum* SR21 showed a salinity tolerance of 0–200 % that of seawater, the optimum being 50–100 % [6]. Thraustochytrids from mangrove environments, where salinity fluctuates highly, may show wider salinity tolerance. “Adeq Precision” measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The value of 25 indicates an adequate signal. To verify the model results, cultivations of *Aurantiochytrium* sp. YLH70 under the optimal condition at flash level were performed in triplicate. The average value in experiments was 36.1 g l<sup>-1</sup> of DCW with 20.5 g l<sup>-1</sup> lipid and 9.03 g l<sup>-1</sup> DHA, very close to the maximum DCW of model prediction (35.5 g l<sup>-1</sup>). There is an improvement of DCW and DHA yields by 149 and 356 %, respectively, relative to those in the initial condition.

### Fermentation Experiments for *Aurantiochytrium* sp. YLH70

Under the optimum culture condition, the time course of DCW, residual reducing sugar concentration, lipid, and DHA yields by *Aurantiochytrium* sp. YLH70 in fed-batch fermentation were shown in Fig. 5. During the 120-h fermentation, an approximate total 180 g l<sup>-1</sup> reducing sugar was consumed. The maximum DCW could reach 78.5 g l<sup>-1</sup> at 114 h, with 51 g l<sup>-1</sup> lipids and 20.1 g l<sup>-1</sup> DHA. The daily DCW and DHA productivities of *Aurantiochytrium* sp. YLH70 could reach up to 16.5 and 4.23 g l<sup>-1</sup> day<sup>-1</sup>, respectively. The most notable characteristics of *Aurantiochytrium* sp. YLH70 is its high growth rate. The daily biomass and DHA productivities under large-scale fermentation were even higher than those at flash level, which would be of great benefit for industrial purpose. The same characteristic was also found in a thraustochytrid-like microorganism 12B [9].

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## Compliance with Ethical Standards

**Conflict of Interest** This study was funded by Zhejiang Provincial Natural Science Foundation of China (no. LQ13C010002), Natural Science Foundation of Zhejiang University of Technology (no. 2013XZ007), and Research Foundation of Zhejiang Education Department (no. Y201225077). The authors declare that they have no conflict of interest.

**Research Involving Human Participants and/or Animals** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

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