Mathematical modeling of fed-batch fermentation of *Schizochytrium* sp. FJU-512 growth and DHA production using a shift control strategy

Mingliang Zhang^{1,2} · Weibin Wu^{1,2} · Xiaolei Guo^{1,2} · You Weichen^{1,2} · Feng Qi^{1,2} · Xianzhang Jiang^{1,2} · Jianzhong Huang^{1,2}

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Abstract

To obtain high-cell-density cultures of *Schizochytrium* sp. FJU-512 for DHA production, two stages of fermentation strategy were used and carbon/nitrogen ratio, DO and temperature were controlled at different levels. The final dry cell weight, total lipid production and DHA yield in 15 l bioreactor reached 103.9, 37.2 and 16.0 g/l, respectively. For the further study of microbial growth and DHA production dynamics, we established a set of kinetic models for the fed-batch production of DHA by *Schizochytrium* sp. FJU-512 in 15 and 100 l fermenters and a compensatory parameter n was integrated into the model in order to find the optimal mathematical equations. A modified Logistic model was proposed to fit the cell growth data and the following kinetic parameters were obtained: $\mu_m = 0.0525/\text{h}$, $X_m = 100 \text{ g/l}$ and $n = 4.1717$ for the 15 l bioreactor, as well as $\mu_m = 0.0382/\text{h}$, $X_m = 107.4371 \text{ g/l}$ and $n = 10$ for the 100 l bioreactor. The Luedeking–Piret equations were utilized to model DHA production, yielding values of $\alpha = 0.0648 \text{ g/g}$ and $\beta = 0.0014 \text{ g/g/h}$ for the 15 l bioreactor, while the values of α and β obtained for the 100 l fermentation were 0.0209 g/g and 0.0030 g/g/h. The predicted results compared with experimental data showed that the established models had a good fitting precision and were able to exactly depict the dynamic features of the DHA production process.

Keywords *Schizochytrium* sp. FJU-512 · Docosahexaenoic acid (DHA) · Fed-batch · Logistic model · Luedeking model

List of symbols

μ_m	Maximum specific growth rate (1/h)
X	Biomass concentration (g/l)
X_m	Maximum biomass concentration (g/l)
P	DHA concentration (g/l)
α	Growth-associated product formation coefficient (g/g)
β	Non-growth-associated product formation coefficient (g/g/h)

Introduction

Docosahexaenoic acid (DHA, C22:6), an omega-3 polyunsaturated fatty acid, is a major structural component of the central nervous system, retina and heart tissue (Ward and Singh 2005), and it has great economic value and widely used in food-related industries (De Swaaf et al. 2003). In fact, DHA also has great potential in medicine and has been investigated in the prevention and treatment of a wide range of diseases, including heart disease, high blood pressure (Meadus et al. 2011), inflammation (Kim and Chung 2007), cancer (Zhuo et al. 2009), and Alzheimer's disease (Lukiw and Bazan 2008).

Marine fungoid protist *Schizochytrium* sp. was known to be an excellent natural DHA producer (Yokochi et al. 1998). However, a great number of variables affect DHA production by *Schizochytrium* sp., such as medium composition (Thyagarajan et al. 2014), nitrogen limitation (Ren et al. 2014), temperature (Unagul et al. 2005) and oxygen supply (Chang et al. 2014), making it difficult to industrial DHA production. Generally, the total cellular lipid can be enhanced under specific cultural conditions, such as low temperature (Zeng

✉ Jianzhong Huang
hjz@fjnu.edu.cn

¹ National Joint Engineering Research Center of Industrial Microbiology and Fermentation Technology, Fujian Normal University, Fuzhou 350108, Fujian, People's Republic of China

² College of Life Sciences, Fujian Normal University, Fuzhou 350108, Fujian, People's Republic of China

et al. 2011), low oxygen levels (Ren et al. 2010) and nitrogen depletion (Sun et al. 2014). However, this nutritional shift down is not a good way since the total biomass and DHA production could be decreased. To circumvent this limitation, we developed a nitrogen limitation, DO-shift and temperature-shift strategy in this work for efficient DHA production by *Schizochytrium* sp. FJU-512. The strategy includes an initial phase with sufficient nitrogen, in combination with high DO and temperature to ensure adequate biomass accumulation. It is useful to introduce a mathematical model to simulate the fermentation process since it can provide very useful information for the further scale-up and implementation of the process. Although many studies have reported the correlation of kinetics of cell growth and mathematical modeling of fermentation processes (Suzuki et al. 1986; Yamane et al. 1997; Panda et al. 1999; Bhattacharya et al. 2015), the kinetics of DHA production by *Schizochytrium* sp. in batch and fed-batch fermentation processes are still quite scarce. For example, Song et al. developed a simple batch fermentation model which uses the logistic equation for growth and the Luedeking–Pirt-like equation for DHA production and substrate consumption by *Schizochytrium limacinum* OUC88 (Song et al. 2010). Surendhiran et al. found that nitrogen was an essential factor for algal growth and they provided kinetic models which simulate the growth and lipid production of two marine microalgae under both nitrogen-replete and nitrogen-depleted conditions, using logistic and Luedeking–Pirt equations (Surendhiran et al. 2015). Thus, it is necessary to investigate the kinetics of pilot-scale fed-batch cultures because the models of high-cell-density fermentations for enhanced DHA production are difficult to control due to the addition of a liquid matrix to the fermentation medium.

In this study, the fermentation kinetics of DHA production by *Schizochytrium* sp. FJU-512 were illustrated in fed-batch process at different scales, and the microbial growth and DHA production dynamics were correlated. Thus, we proposed a modified model that employs the logistic equation for growth and the Luedeking–Pirt-like equations for DHA production.

Methods

Microorganism

Schizochytrium sp. FJU-512 was used for DHA production. It was originally isolated using the pine pollen baiting technique.

Medium

A basal medium comprising 30 g/l glucose, 10 g/l peptone, 5 g/l yeast extract and 15 g/l sea salts was used for routine cultivation. The fermentation medium contained 30 g/l of glucose, 2.5 g/l $(\text{NH}_4)_2\text{SO}_4$, 15 g/l yeast extract, 15 g/l peptone, 25 g/l sea salts, 2 g/l KH_2PO_4 , 3 g/l Na_2SO_4 , 0.005 g/l vitamin B₁, and 0.005 g/l vitamin B₁₂.

Inoculum preparation

Static cultures of *Schizochytrium* sp. FJU-512 were grown for 2 days at 28 °C and used to inoculate 1 l flasks containing 400 ml of basal medium. The resulting cultures were incubated at 28 °C under constant orbital shaking at 230 rpm for 2 days.

Fed-batch cultures

The fed-batch cultures were carried out in a 15 and a 100 l bioreactor (Zhenjiang East Biotech Equipment and Technology Co., Ltd., China), respectively. Culture conditions such as temperature, pH, DO and agitation rate were controlled automatically. We started the cultivation with initial volumes of 8 l in the 15 l fermenter and 55 l in the 100 l fermenter. Subsequently, 10 g/l organic nitrogen source (7 g/l peptone and 3 g/l yeast extract), 20 g/l sea salts and 80% glucose were added continuously into the fermenters when the residual glucose was below 2% after 24 h. Fed-batch cultures were performed at 28 °C in bioreactors before mid-exponential phase, after which the temperature was lowered to below 28 °C to enhance the accumulation of lipids. The initial aeration rate was 1 vvm and was subsequently adjusted manually. pH was maintained at 5.7 ± 0.1 by automated addition of 28% industrial ammonia.

Biomass determination

A 10 ml cell suspension sample was taken from the culture and centrifuged at 8000 rpm for 5 min. The cell pellets were rinsed with deionized water and centrifuged under the same conditions. This procedure was repeated 2–3 times. The washed cells were dried at 85 °C for 24 h to obtain the dry biomass used to determine the cell dry weight.

Lipid extraction

Dried cell samples prepared same as above were used directly for lipid extraction. The cell pellets were dissolved in 5 ml 6 M HCl and incubated for 1 h at 80 °C, after which the lipids were extracted using 2 ml *n*-hexane and 0.75 ml absolute ethyl

alcohol. The mixture was blended by vigorous vortexing and centrifuged at 800×g for 2 min to improve phase separation. The extraction process was repeated 3 times to collect the greatest possible amount of lipids. The total lipids were determined gravimetrically after evaporation of the solvents under oxygen-free nitrogen on a heating block maintained at 50 °C.

Analysis of fatty acid composition

An aliquot comprising 100 mg of lipids was transmethylated at 62 °C for 1 h using 2 ml 10% (v/v) methanolic HCl. Subsequently, the fatty acid methyl esters (FAMES) were dissolved in 2 ml *n*-hexane. GC–MS (Agilent 6890N/5975C) analysis of FAMES was conducted using an HP-INNOWAX capillary column (30 m×0.25 mm×0.25 µm). High purity helium (99.999%) was used as the carrier gas at a flow rate of 1.0 ml/min. The injector temperature was maintained at 250 °C, and a volume of 1 µl was injected using the split mode at a ratio of 5:1. Column temperature was raised from 150 to 220 °C at 10 °C/min and further to 230 °C at 2 °C/min, where it was kept for 5 min. All fatty acids were identified by comparing to the NIST mass spectral library (Agilent Technologies, USA). The mass spectroscopy instrument was operated at an ionization voltage of 70 eV, with a scan range of 35–450 amu. Concentrations of the fatty acids were calculated based on the total peak areas compared to an internal standard, as published previously (Huang et al. 2003).

Defining the kinetic parameters

To prevent the inhibitory effects of high glucose concentrations in the cultivation process and obtain maximum DHA production, we chose fed-batch cultures to obtain kinetics, estimate models, and calculate parameters. Kinetic parameters were calculated according to the following equations:

$$\frac{dX}{dt} = \mu_m X \left[1 - \left(\frac{X}{X_m} \right)^n \right], \quad (1)$$

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X. \quad (2)$$

The logistic constants for cell growth were evaluated using the cftool kit in MATLAB version 7.1. Equations 1 and 2 were solved by numerical integration and ode45 solver version was employed for fitting of the results.

Results and discussion

Effects of fermentation conditions on *Schizochytrium* sp. growth and DHA production

In general, glucose was utilized by *Schizochytrium* to produce DHA under oxygen-limiting and nitrogen-limiting conditions. We performed experiments in several stages to increase the biomass concentration and promote DHA production. The fermentation process of *Schizochytrium* can be divided into two stages (Chi et al. 2009): stage 1, increase of the biomass with little lipids accumulation; stage 2, no accumulation of biomass with enlarged cell size due to lipids and DHA synthesis. At first, the cells grew rapidly with fast glucose consumption under adequate nitrogen and oxygen supply, whereby the dissolved oxygen tension (DOT) was maintained above 20% of air saturation between 0 and 48 h by manually controlling the stirrer speed (200–465 rpm) and aeration rate (air volume/culture volume/min: 1–2 vvm). The pH was kept at 5.7 ± 0.1 by automated addition of 28% industrial ammonia at the first 60 h, and the cultivation temperature was set to 28 °C during the first 96 h. In the second stage, the cells expanded as intracellular lipids accumulated, but the biomass did not increase much until the end of fermentation. It is known that a high carbon/nitrogen ratio, low DO and low temperature are essential for lipid accumulation and DHA production in oleaginous microorganisms (Zeng et al. 2011; Ren et al. 2010; Sun et al. 2014). Therefore, it is not necessary to adjust the agitation and aeration rates, and DO was limited within 5%. Subsequently, 80% glucose and 10 g/l organic nitrogen were added continuously to ensure an excess supply of carbon source and nitrogen without pH regulation. After 96 h, the temperature was decreased from 28 to 25 °C to enhance DHA accumulation. The results of this control strategy revealed that DHA content increased from 44.1 to 51.5% with reduced DO after 36 h (Table 1). When available carbon was constantly increased and nitrogen was limited from 60 h to the end of fermentation, the lipid content increased from 25.8 to 29.4%. DHA productivity was 133.7 mg/l/h in the fed-batch fermentation after 120 h, and DHA content increased from 42.2 to 44.7% with changing cultivation temperature. The DHA concentration experienced a slight decrease when we reduced the temperature (Table 1). The highest cumulative production at 120 h was achieved using the feeding strategy, and DCW, total yield and DHA production reaching 103.9, 37.2 and 16.0 g/l, respectively (Table 1).

Thus, the results obtained in the present study indicate that DHA production by *Schizochytrium* sp. FJU-512 is greatly influenced by the availability of nitrogen. Although

Table 1 Biomass, TFA and DHA contents obtained in the fed-batch culture of *Schizochytrium* sp. FJU-512 in the 15 l bioreactor

Time (h)	Biomass	Total lipids (g/l)	DHA (g/l)	TFA (%)	DHA (%)
36.00	13.67 ± 0.82	2.47 ± 0.12	1.09 ± 0.05	18.07 ± 0.41	44.13 ± 0.63
48.00	39.16 ± 2.35	8.72 ± 0.44	4.49 ± 0.22	22.27 ± 0.57	51.49 ± 0.91
60.00	64.50 ± 2.87	16.65 ± 0.96	7.92 ± 0.43	25.81 ± 1.29	47.57 ± 1.50
72.00	76.40 ± 3.58	22.44 ± 1.12	10.57 ± 0.53	29.37 ± 0.11	47.10 ± 1.72
84.00	88.22 ± 3.29	25.92 ± 1.30	10.70 ± 0.53	29.38 ± 0.93	41.28 ± 0.82
90.00	88.40 ± 3.30	27.04 ± 1.35	11.68 ± 0.58	30.59 ± 1.67	43.20 ± 1.65
96.00	88.72 ± 2.32	31.16 ± 0.96	13.16 ± 0.66	35.12 ± 0.57	42.23 ± 0.91
108.00	96.36 ± 3.78	32.88 ± 1.14	14.71 ± 0.74	34.12 ± 1.29	44.74 ± 1.50
120.00	103.86 ± 3.23	37.18 ± 0.86	16.04 ± 0.80	35.80 ± 0.84	43.14 ± 0.60

nitrogen limitation is a prerequisite for lipid accumulation in oleaginous microorganisms, a high C/N ratio leads to a decrease in biomass (Huang et al. 2003). The regulation of lipid biosynthesis in oleaginous microorganisms requires a sufficient supply of NADPH as reductant, while under conditions of nitrogen exhaustion the activity of AMP deaminase is up-regulated, resulting in the formation of acetyl-CoA (Ratledge 2004). Surendhiran et al. reported that under nitrogen-limited conditions, the lipid content of microalgae increased by shifting the metabolic fluxes from the protein and carbohydrate pathways (Surendhiran et al. 2015).

In addition, temperature was another main factor that affected the accumulation of biomass and DHA. Zeng et al. obtained the highest DHA content of 51.98% of total fatty acids and DHA production of 6.05% (per dry cell weight) in *Schizochytrium* HX-308, using the strategy of shifting the cultivation temperature from 30 °C for 32 h to 20 °C for 12 h in shake flasks (Zeng et al. 2011). Pasquet et al. found that microalgal cell membrane fluidity increases as an adaptation to low temperatures (Pasquet et al. 2014). Furthermore, it has been reported that only the intracellular lipids and organelle membranes were influenced by the cultivation temperature (Chodchoey and Verduyn 2012). In this work, we used a temperature shift from 28 to 25 °C. In the first stage, the culture temperature was set at 28 °C in order to generate sufficient biomass. In the lipid accumulation stage, the temperature was lowered to 25 °C, and the DHA content increased significantly, even though the cells grew slowly. Thus, this simple temperature shift strategy is an effective way to obtain enhanced biomass and DHA accumulation from *Schizochytrium* sp. FJU-512.

Furthermore, a high oxygen supply does not necessarily result in more DHA production in *Schizochytrium* sp. Qu et al. showed that an increased KLa of 150/h provided conditions favorable for cell growth, while the biosynthesis of DHA could be effectively improved as the KLa was controlled at 88.5/h (Qu et al. 2011). It is known that *Schizochytrium* sp. uses a PKS-like system to synthesize PUFAs, in which acetyl-CoA and malonyl-CoA are utilized as the

initial building blocks (Metz et al. 2001). An anaerobic culture system was needed for DHA synthesis from saturated lipids in oleaginous microorganisms. Thus, a strategy of shifting the growth conditions from high to low oxygen concentration was developed to increase the DHA production in this study. Using fed-batch culture combined with a temperature and oxygen-concentration shift strategy, *Schizochytrium* sp. FJU-512 grown in the 15 l-reactor reached a biomass of 103.9 g/l, a total lipid yield of 37.2 g/l, and a DHA yield of 16.0 g/l. By contrast, the highest biomass, lipid and DHA concentrations obtained during fermentation in the 100 l reactor were 110.8, 50.8 and 22.3 g/l, respectively. Thus, both the temperature and oxygen shift strategies have been demonstrated as effective methods to enhance DHA production by *Schizochytrium* sp. FJU-512 in 15 and 100 l bioreactors.

Microbial growth

According to a study by Song et al., DHA synthesis is associated with the microbial growth rate (Qu et al. 2013). Qu et al. indicated that fed-batch cultivation was a more efficient manipulation compared to batch fermentation (Gaden 2000). A simple kinetic and logistic equation was given in this study to describe the cell growth of *Schizochytrium* sp. FJU-512 in the fed-batch process using MATLAB 7.1. Time courses of cell growth (Figs. 1, 2) were plotted by fitting sigmoidal growth data to the integrated equation. To study the kinetics and obtain a good fitting effect of the model, some assumptions were required: (1) the concentration of glucose was high enough for cell growth and DHA biosynthesis; (2) the dissolved oxygen was sufficient and was not a limiting factor for the growth rate during the entire fermentation process; (3) the feeding volume was equal to the volume of evaporation and sample withdrawal so that the biosynthetic process could be regarded as constant; (4) the similarity of structure and dimensions of the experimental fermenters (heat and mass transfer coefficients) are consistent. However, there were large differences between the 15 and 100 l stirred tank bioreactors. Here, the parameter n

Fig. 1 Fitting of the growth curve measured in the in 15 l fermenter with the modified Logistic model

Fig. 2 Fitting of the growth curve measured in the in 100 l fermenter with the modified Logistic model

was used to add model compensation in order to obtain the optimal mathematical equations.

Interestingly, a lag phase was observed at 24–36 h in both the 15 and the 100 l fermenter, suggesting that biomass accumulation was inhibited by the substrate. However, carbon and nitrogen sources were consumed quickly during the exponential growth phase. Thus, the concentrated medium was added after the lag phase comprised 80% glucose and 10 g/l nitrogen source, in order to avoid unnecessary dilution. Subsequently, the cell growth kinetics of *Schizochytrium* sp. FJU-512 during fed-batch cultivation and the multi-stage control strategy in the 15 and 100 l fermenters were analyzed and simulated in accordance with the results. The integrated logistic model was established to fit the cell growth kinetics. The value of μ_m was 0.0525/h, X_m

was 100 g/l and n was 4.1717. These values were obtained from the parameter estimation with a relative error of 0.1160 in the 15 l bioreactor. Correspondingly, μ_m , X_m and n were 0.0382/h, 107.4371 g/l and 10, respectively, with a relative error of 0.0810 in the 100 l fermenter. The value of μ_m in the 15 l fermentation process was less than that of the 100 l cultivation mainly because there was a much longer time lag (36 h) in the 100 l fed-batch culture than in the 15 l fermenter (24 h). The X_m of the 100 l fermenter was greater than that of 15 l, which was attributed mainly to the better efficiency of dissolved oxygen transfer in the larger fermentation tank. It was, therefore, assumed that the growth follows logistic kinetics as follows:

$$\frac{dX}{dt} = 0.0525X \left[1 - \left(\frac{X}{100} \right)^{4.1717} \right], \quad (3)$$

$$\frac{dX}{dt} = 0.0382X \left[1 - \left(\frac{X}{107.4371} \right)^{10} \right]. \quad (4)$$

DHA production

The relationships between the rate of product formation and cell growth can be divided into three types: the growth-associated model, semi-growth-associated model and no-growth-associated model (Gaden 2000). In this case, increased DHA production was associated with enhanced lipid and biomass accumulation. The results thus indicated that DHA production could be considered as a semi-growth-associated process. Figures 3 and 4 show the kinetics of the DHA yield in the 15 and 100 l fed-batch reactors, respectively, based on the Luedeking–Piret model. The parameters α and β for the cultivation in the 15 l stirred tank obtained using the model were 0.0648 g/g and 0.0014 g/g/h, respectively. The final

Fig. 3 Fitting of the DHA yield curve measured in the in 15 l fermenter with the modified Luedeking–Piret model

Fig. 4 Fitting of the DHA yield curve measured in the 100 l fermenter with the modified Luedeking–Piret model

formula describing the DHA production kinetics in the 15 l bioreactor was:

$$\frac{dP}{dt} = 0.003402X \left[1 - \left(\frac{X}{100} \right)^{4.1717} \right] + 0.0014X. \quad (5)$$

By contrast, the values of α and β obtained for the 100 l fermentation were 0.0209 g/g and 0.0030 g/g/h, respectively. The corresponding DHA production model was described as:

$$\frac{dP}{dt} = 0.00079838X \left[1 - \left(\frac{X}{107.4371} \right)^{10} \right] + 0.0030X. \quad (6)$$

Therefore, the results demonstrate that the value of α was much higher than β in each case, suggesting that cell growth has a major effect on DHA production in *Schizochytrium* sp. FJU-512.

Conclusions

Our results demonstrate that the culture-condition shift strategy developed in this study provides an effective method to enhance DHA production by *Schizochytrium* sp., even in scaled-up processes. The μ_m value in the 15 l fed-batch fermenter was higher than that of the 100 l reactor while the X_m value showed the opposite trend. Both values had been estimated as the same levels, which was mainly due to the modification of the parameter n . The relevant errors were 11.60 and 8.10%, respectively, indicating that the modified logistic and Luedeking–Piret model equations contributed to provide a satisfactory fitting for the kinetics of DHA production by *Schizochytrium* sp. FJU-512 in fed-batch systems

at different scales. The fitting results also revealed that the parameter α was greater than β , which demonstrated that DHA production was highly dependent on cells growth, in agreement with the previous report (Surendhiran et al. 2015). Furthermore, the established models had a good fitting precision and were used to exactly depict the dynamic features of the DHA production process of *Schizochytrium* sp. FJU-512. Therefore, in this study we provide a useful analysis tool for optimization of biomass accumulation and DHA production of *Schizochytrium* sp. through the mathematical modeling strategy of fermentation process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

References

- Bhattacharya M, Guchhait S, Biswas D, Datta S (2015) Waste lubricating oil removal in a batch reactor by mixed bacterial consortium: a kinetic study. *Bioprocess Biosyst Eng* 38(11):2095–2106. <https://doi.org/10.1007/s00449-015-1449-9>
- Chang G, Wu J, Jiang C, Tian G, Wu Q, Chang M, Wang X (2014) The relationship of oxygen uptake rate and $k(L)a$ with rheological properties in high cell density cultivation of docosahexaenoic acid by *Schizochytrium* sp. S31. *Bioresour Technol* 152:234–240. <https://doi.org/10.1016/j.biortech.2013.11.002>
- Chi ZY, Liu Y, Frear C, Chen SL (2009) Study of a two-stage growth of DHA-producing marine algae *Schizochytrium limacinum* SR21 with shifting dissolved oxygen level. *Appl Microbiol Biotechnol* 81(6):1141–1148. <https://doi.org/10.1007/s00253-008-1740-7>
- Chodchoey K, Verduyn C (2012) Growth, fatty acid profile in major lipid classes and lipid fluidity of *Aurantiochytrium mangrovei* Sk-02 as a function of growth temperature. *Braz J Microbiol* 43(1):187–200. <https://doi.org/10.1590/S1517-838220120001000020>
- De Swaaf ME, Sijtsma L, Pronk JT (2003) High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Cryptocodinium cohnii*. *Biotechnol Bioeng* 81(6):666–672. <https://doi.org/10.1002/bit.10513>
- Gaden EL (2000) Fermentation process kinetics (Reprinted from J of Biochemical and Microbiological Technology and Engineering, vol 1, pg 413, 1959). *Biotechnol Bioeng* 67(6):629–635. [https://doi.org/10.1002/\(sici\)1097-0290\(20000320\)67:6<629::aid-bit2>3.0.co;2-p](https://doi.org/10.1002/(sici)1097-0290(20000320)67:6<629::aid-bit2>3.0.co;2-p)
- Huang JZ, Aki T, Yokochi T, Nakahara T, Honda D, Kawamoto S, Shigeta S, Ono K, Suzuki O (2003) Grouping newly isolated docosahexaenoic acid-producing thraustochytrids based on their polyunsaturated fatty acid profiles and comparative analysis of 18S rRNA genes. *Mar Biotechnol* 5(5):450–457. <https://doi.org/10.1007/s10126-002-0110-1>
- Kim YJ, Chung HY (2007) Antioxidative and anti-inflammatory actions of docosahexaenoic acid and eicosapentaenoic acid in

- renal epithelial cells and macrophages. *J Med Food* 10(2):225–231. <https://doi.org/10.1089/jmf.2006.092>
- Lukiw WJ, Bazan NG (2008) Docosahexaenoic acid and the aging brain. *J Nutr* 138(12):2510–2514. <https://doi.org/10.3945/jn.108.096016>
- Meadus WJ, Duff P, Rolland D, Aalhus JL, Uttaro B, Dugan MER (2011) Feeding docosahexaenoic acid to pigs reduces blood triglycerides and induces gene expression for fat oxidation. *Can J Anim Sci* 91(4):601–612. <https://doi.org/10.4141/cjas2011-055>
- Metz JG, Roessler P, Facciotti D, Levering C, Dittrich F, Lassner M, Valentine R, Lardizabal K, Domergue F, Yamada A, Yazawa K, Knauf V, Browse J (2001) Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science* 293(5528):290–293. <https://doi.org/10.1126/science.1059593>
- Panda AK, Khan RH, Rao KB, Totey SM (1999) Kinetics of inclusion body production in batch and high cell density fed-batch culture of *Escherichia coli* expressing ovine growth hormone. *J Biotechnol* 75(2–3):161–172. [https://doi.org/10.1016/s0168-1656\(99\)00157-1](https://doi.org/10.1016/s0168-1656(99)00157-1)
- Pasquet V, Ulmann L, Mimouni V, Guiheneuf F, Jacqueline B, Morant-Manceau A, Tremblin G (2014) Fatty acids profile and temperature in the cultured marine diatom *Odontella aurita*. *J Appl Phycol* 26(6):2265–2271. <https://doi.org/10.1007/s10811-014-0252-3>
- Qu L, Ji XJ, Ren LJ, Nie ZK, Feng Y, Wu WJ, Ouyang PK, Huang H (2011) Enhancement of docosahexaenoic acid production by *Schizochytrium* sp. using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. *Lett Appl Microbiol* 52(1):22–27. <https://doi.org/10.1111/j.1472-765X.2010.02960.x>
- Qu L, Ren L-J, Sun G-N, Ji X-J, Nie Z-K, Huang H (2013) Batch, fed-batch and repeated fed-batch fermentation processes of the marine thraustochytrid *Schizochytrium* sp for producing docosahexaenoic acid. *Bioprocess Biosyst Eng* 36(12):1905–1912. <https://doi.org/10.1007/s00449-013-0966-7>
- Ratledge C (2004) Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie* 86(11):807–815. <https://doi.org/10.1016/j.biochi.2004.09.017>
- Ren LJ, Ji XJ, Huang H, Qu L, Feng Y, Tong QQ, Ouyang PK (2010) Development of a stepwise aeration control strategy for efficient docosahexaenoic acid production by *Schizochytrium* sp. *Appl Microbiol Biotechnol* 87(5):1649–1656. <https://doi.org/10.1007/s00253-010-2639-7>
- Ren L-J, Sun L-N, Zhuang X-Y, Qu L, Ji X-J, Huang H (2014) Regulation of docosahexaenoic acid production by *Schizochytrium* sp.: effect of nitrogen addition. *Bioprocess Biosyst Eng* 37(5):865–872. <https://doi.org/10.1007/s00449-013-1057-5>
- Song XJ, Zhang XC, Kuang CH, Zhu LY, Zhao XB (2010) Batch kinetics and modeling of DHA production by *S. limacinum* OUC88. *Food Bioprod Process* 88(C1):26–30. <https://doi.org/10.1016/j.fbp.2009.12.004>
- Sun L, Ren L, Zhuang X, Ji X, Yan J, Huang H (2014) Differential effects of nutrient limitations on biochemical constituents and docosahexaenoic acid production of *Schizochytrium* sp. *Biore-sour Technol* 159:199–206. <https://doi.org/10.1016/j.biortech.2014.02.106>
- Surendhiran D, Vijay M, Sivaprakash B, Sirajunnisa A (2015) Kinetic modeling of microalgal growth and lipid synthesis for biodiesel production. *3 Biotech* 5(5):663–669. <https://doi.org/10.1007/s13205-014-0264-3>
- Suzuki T, Yamane T, Shimizu S (1986) Kinetics and effect of nitrogen source feeding on production of poly- β -hydroxybutyric acid by fed-batch culture. *Appl Microbiol Biotechnol* 24(5):366–369. <https://doi.org/10.1007/bf00294591>
- Thyagarajan T, Puri M, Vongsivut J, Barrow CJ (2014) Evaluation of bread crumbs as a potential carbon source for the growth of thraustochytrid species for oil and omega-3 production. *Nutrients* 6(5):2104–2114. <https://doi.org/10.3390/nu6052104>
- Unagul P, Assantachai C, Phadungruengluij S, Suphantharika M, Verduyn C (2005) Properties of the docosahexaenoic acid-producer *Schizochytrium mangrovei* Sk-02: effects of glucose, temperature and salinity and their interaction. *Bot Mar* 48(5–6):387–394. <https://doi.org/10.1515/bot.2005.052>
- Ward OP, Singh A (2005) Omega-3/6 fatty acids: alternative sources of production. *Process Biochem* 40(12):3627–3652. <https://doi.org/10.1016/j.procbio.2005.02.020>
- Yamane Y, Higashida K, Nakashimada Y, Kakizono T, Nishio N (1997) Influence of oxygen and glucose on primary metabolism and astaxanthin production by *Phaffia rhodozyma* in batch and fed-batch cultures: kinetic and stoichiometric analysis. *Appl Environ Microbiol* 63(11):4471–4478
- Yokochi T, Honda D, Higashihara T, Nakahara T (1998) Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. *Appl Microbiol Biotechnol* 49(1):72–76. <https://doi.org/10.1007/s002530051139>
- Zeng Y, Ji XJ, Lian M, Ren LJ, Jin LJ, Ouyang PK, Huang H (2011) Development of a temperature shift strategy for efficient docosahexaenoic acid production by a marine fungoid protist, *Schizochytrium* sp. HX-308. *Appl Biochem Biotechnol* 164(3):249–255. <https://doi.org/10.1007/s12010-010-9131-9>
- Zhuo Z, Zhang L, Mu Q, Lou Y, Gong Z, Shi Y, Ouyang G, Zhang Y (2009) The effect of combination treatment with docosahexaenoic acid and 5-fluorouracil on the mRNA expression of apoptosis-related genes, including the novel gene BCL2L12, in gastric cancer cells. *Vitro Cell Dev Biol Anim* 45(1–2):69–74. <https://doi.org/10.1007/s11626-008-9154-5>