Improving docosahexaenoic acid productivity of Schizochytrium sp. by a two-stage AEMR/shake mixed culture mode

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HIGHLIGHTS
- Aeration-enhanced membrane reactor was used to increase dissolved oxygen in the fermentation broth.
- A two-stage mixed culture mode was proposed to improve the DHA productivity of Schizochytrium sp.
- Culture time is shortened and maximum DHA productivity is enhanced.

ABSTRACT
In this work, an aeration-enhanced membrane reactor (AEMR) was built to control dissolved oxygen in Schizochytrium sp. broth. The effect of culture modes, i.e. single shake and AEMR mode, on the docosahexaenoic acid (DHA) productivity of Schizochytrium sp. was investigated. Experimental results showed that the biomass production in the AEMR mode was higher than that in the single shake mode, while the final DHA productivity in single shake mode was higher than that in the AEMR mode. A two-stage mixed culture mode was proposed, in which Schizochytrium sp. was cultured in AEMR mode at a flow rate of 0.2 L min⁻¹ for 60 h to first increase biomass production, and then shifted to shake mode to improve DHA productivity. Compared to the single shake mode, the DHA productivity in mixed mode costed 40% less culture time and the DHA productivity at 96 h exhibited a relative increase of 60%.

1. Introduction
Docosahexaenoic acid (DHA) plays a significant role in human cognitive ability and brain physiology, including the structural growth, functional development and maintenance of the brain (Nettleton, 1993). Although the demand for DHA in medical care is considerable, the production of DHA is quite limited and mainly derived from sea fish. Moreover, many important issues exist in the production of DHA from sea fish, including fluctuant throughput, potential enriched injurant in the fish oil, difficulty to in removing the fishy taste (Belardi et al., 2000).

Two stages are involved in the fermentation of Schizochytrium sp., cell proliferation and lipid accumulation. During these two stages in fermentation, the nutrient substance demand and the parameter of fermentation operation are quite different: the cell proliferation consumes massive carbon and nitrogen resources, while lipid accumulation mainly occurs under a condition called nitrogen hunger (Ratledge, 2004). Hence sufficient carbon resource and limited nitrogen resource should be responsible for lipid accumulation. Additionally, as Schizochytrium sp. is heterotrophism aerobic microbe, the dissolved oxygen (DO) has enormous implications on cell proliferation and lipid accumulation. Others considering Schizochytrium limacium SR21 reported that cells multiplied and cell density increased with constant cell size and weight under high DO, while microbial oil accumulated with an obvious volume gain under low DO (Chi et al., 2009). Other research showed that the oil and DHA percentage increased with low DO (Bailey et al., 2003). The effects of nitrogen resources and DO on the DHA accumulation of Aurantiochytrium sp. T66 were also investigated, and it was found DHA percentage accounted for 29% with restricted nitrogen resources and then increased to 52% when DO was restricted (Jakobsen et al., 2008). Therefore, DO seemed to be a significant factor in DHA accumulation.

In this paper, polypropylene hollow fiber membrane was mounted in a shake flask to enhance aeration and increase DO,
working as a membrane reactor. Compared to the shake culture, the aeration-enhanced membrane bioreactor (AEMR) culture can increase the concentration of DO and facilitate cell proliferation. Furthermore, a novel cultivation process, two-stage AEMR/shake mixed culture mode, was proposed to combine the advantages of both modes.

2. Methods

2.1. Materials

Schizochytrium sp. (ATCC 20888) was used and the membrane module was prepared by four 10 cm long membrane fibers (Enka, pore size 0.20 μm) with the both ends inserted into a glass pipe and fixed by epoxy. Yeast extract was purchased from OXOID Ltd., UK. If not specifically mentioned, all other chemicals were provided by Sinopharm Chemical Reagent Co. Ltd., China.

2.2. Cultivation of cells

Seed broth: 30 g/L glucose, 10 g/L peptone, 5 g/L yeast extract and 20 g/L deep sea crystal (pH = 7). Fermentation broth: 70 g/L glucose, 15 g/L peptone and 20 g/L deep sea crystal (pH = 7).

The cells were activated in the seed broth with stored cells and incubated at 25 °C and 170 rpm for 48 h. 2.5 mL of activated seed cell solution was added to 50 mL seed broth in a 250 mL flask and incubated at 25 °C and 170 rpm for 36 h. The cell suspension was used as inoculum for later studies.

Cultivation for fermentation: (a) shake mode: 5 mL of seed was added to 250 mL flasks containing 100 mL of fermentation broth and incubated at 25 °C and 170 rpm for 144 h or more. (b) AEMR mode: 5 mL of seed was added into 250 mL flasks containing 100 mL of fermentation broth, and standard sterile air was bubbled into the fermentation broth through membrane module at different flow rates (0.1, 0.2 and 0.3 L min⁻¹) and incubated at 25 °C and 170 rpm for 144 h.

2.3. Measurement for kinetics of cell growth

The biomass and lipid concentrations were measured by a classical method and the DHA percentage was detected by chromatography internal standard method as described in the supporting information.

3. Results and discussion

3.1. Effect of single culture mode on the fermentation

Fig. 1a shows the variation in the trend of biomass and lipid amount vs. time. During the initial stage (<72 h), although the biomass and lipid amount apparently increased in both flask and AEMR culture processes, the biomass in the AEMR mode obviously surpassed that of the shake mode and the increase in lipid amount was quite small, indicating that the AEMR mode was conducive to biomass accumulation in the initial incubation stage. During the latter stage (>72 h), the biomass and lipid amount in the shake mode increased till 192 h and then reached a plateau stage as shown in Fig. S1 (Supporting information). However, the biomass and lipid amount in the AEMR mode stopped or decreased with the different air flow rates.

The residual glucose concentration has good correspondence with the accumulation of biomass, as shown in Fig. S2. In the initial stage, cells in the AEMR consumed more glucose, especially at the flow rate of 0.2 L min⁻¹. However in the later stage, less glucose was consumed in the AEMR mode while slightly more glucose was consumed in the shake mode.

The variation in lipid and DHA percentage with culture time are plotted in Fig. 1b. The lipid and DHA percentage in the shake mode was bigger than that in the AEMR culture. Generally, the biomass includes two parts: essential substance for proliferation and self-subsist and non-essential substance, here lipid or DHA. For the initial stage, the biomass and lipid amounts in the AEMR culture obviously exceeded that in the shake mode, while the lipid content and DHA percentage in the AEMR culture were smaller than that in the shake mode, indicating that the AEMR culture is prone to increasing cell proliferation rather than lipid and DHA accumulation.

In fact, the AEMR culture supplied more air to the broth in the form of small bubbles, which has a considerable specific surface, thus increasing the DO (Gabelman and Hwang, 1999). Therefore, a higher air flow rate brings about greater DO in the broth, as shown in Fig. 2a.

The air flow rate is an important factor that affects the accumulation of biomass and lipid in the AEMR culture. In the early stage of the AEMR culture, a high flow rate, i.e. high DO, led to an obvious biomass improvement (Fig. 1a), while a low flow rate, i.e. low DO, led to a relatively small biomass but a higher lipid and DHA percentage (Fig. 1b), which is consistent with the report by Chi et al. (2009). Moreover, excessive DO may cause potential damage to cells, resulting in a decrease in biomass and lipid amount in the latter stage. Considering the dual effect of DO, 0.2 L min⁻¹ is an appropriate choice for the accumulation of biomass in the initial stage.
Fig. 1b shows that in the later stage of fermentation the lipid and DHA percentage gradually approximated to a stable plateau, while Fig. 2b shows that DHA productivity was kept relatively high in the shake mode compared to that in the AEMR culture. This also can be explained as a result of the relatively low DO in the shake mode, because a low DO favors the accumulation of lipid and DHA (Bailey et al., 2003; Chi et al., 2009).

### 3.2. Effect of mixed culture mode on fermentation

Considering the different effects of single shake and AEMR cultures on the fermentation of *Schizochytrium* sp., a two-stage mixed culture mode was proposed, increasing the biomass with an AEMR culture and then accumulating the lipid and DHA by the shake culture. An appropriate air flow rate of 0.2 L min$^{-1}$ was selected for further study. Three shift points were considered as shown in Fig. 3.

Shifting to the shake mode after 48 h, 60 h and 72 h in the AEMR mode, both biomass and lipid concentration showed a sustainable growth of *Schizochytrium* sp. Compared to the single culture modes, of either shake culture or AEMR culture, the two-stage mixed mode provided a higher lipid amount. And the lipid content in the mixed mode can approach 60%, which is bigger than previous report after optimization (Bailey et al., 2003; Gauza et al., 2008).

After the culture shift, the DHA productivity also showed an improvement compared to the single mode (Fig. 4). In the mixed mode, the DHA productivity is maximum when shifted at 60 h, indicating that the best culture mode to enrich DHA is firstly culturing in AEMR to boost the biomass for 60 h and then transferring to shake culture to enrich DHA. The maximum DHA productivity of 16.35 mg L$^{-1}$ h$^{-1}$ in the single shake mode consumed 120 h, while the same DHA productivity in the mixed mode consumed less than 72 h, saving 40% in culture time. For the same culture time of 96 h, DHA productivity in single shake mode and mixed mode were 13.5 mg L$^{-1}$ h$^{-1}$ and 21.26 mg L$^{-1}$ h$^{-1}$, respectively, and the DHA productivity for a certain period of time is increased by 60%. Additionally, the maximum DHA productivity in mixed mode is 30%
higher than that in single shake mode, which is a quite remarkable improvement compared to previous report on a stepwise aeration control strategy (Ren et al., 2009b). Other strategies were also proposed to increase the DHA production by reinforcing acetyl-CoA or NADPH supply, but they did not take the time and efficiency into account (Ren et al., 2009a). Genetically engineering techniques were also adopted to improve the DHA production (Lian et al., 2010), and the DHA percentage of the total fatty acids could be increased 40% higher than the wild strain. Obvious differences existed between the reports on the DHA productivity as listed in Table 1, and this probably roots in the diversity of Schizochytrium sp. strains and the adoption of the culture modes. Considering the mixed mode, it is potential to obtain a significant increase in the DHA productivity after nutrition optimization and genetic modification. Our effective and efficient AEMR mode is expected to facilitate the cultivation of other microbial, especially for the shake-sensitive strains.

4. Conclusions

AEMR mode is conducive to the growth of biomass, while the shake mode is beneficial to lipid accumulation. A two-stage mixed culture mode was proposed to boost the biomass by the AEMR mode in a first stage and then enrich the lipid and DHA by the shake mode in a second stage. In the mixed mode, lipid content and DHA productivity were greatly enhanced and the maximum DHA production was 30% higher than that in the shake mode. The mixed mode would provide guidance for industrial production of DHA and the other single cell oil production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.05.072.

References


