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The relationship of oxygen uptake rate and k_La with rheological properties in high cell density cultivation of docosahexaenoic acid by *Schizochytrium* sp. S31

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HIGHLIGHTS

- DO limitation was commonly encountered in high cell density cultivation of DHA.
- The cultures with more than 100 g/L cell density exhibited shear-thinning behavior.
- Apparent viscosity of the broth significantly affected the change of k_La.
- OUR was proposed to be an on-line parameter for scale-up of DHA production.

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ABSTRACT

Three independent cultures by fed batch strategy under different oxygen supply levels were investigated with *Schizochytrium* sp. S31 on glycerol in 50 L bioreactor. Three cultures all achieved high cell density cultivation (HCDC) with more than 100 g/L biomass density. However, the culture with middle oxygen supply level achieved the highest DHA concentration at 21.26 g/L. Dissolved oxygen (DO) limitation was commonly encountered in the present cultures, which was due to the dramatic decrease of k_La in high oxygen supply culture resulted from significantly increasing apparent viscosity of the broth. The rheological properties of the three cultures all exhibited shear-thinning behavior. The oxygen uptake rate (OUR) predominately influenced by k_La was suggested to replace DO as on-line control parameter for scale-up production of DHA.

1. Introduction

Docosahexaenoic acid (DHA, 22:6) has received wide attention due to its conspicuously nutritional effect to infant, children and adults (Ratledge, 2004; Sinclair et al., 2005). Currently, DHA production by heterotrophic microalgae species such as *Crypthecodinium cohnii*, *Schizchytrium* and *Ulkenia* have been commercialized (Ratledge, 2004). Among these strains, *Schizochytrium* sp., a thraustochytrid in the kingdom of Stramenopila, could achieve high cell density cultivation (HCDC) with dry biomass density more than 100 g/L on glucose (Bailey et al., 2003) or glycerol (Chang et al., 2013a; Chi et al., 2009a).

Many researchers have proved that low dissolved oxygen (DO) was favored for DHA production (Bailey et al., 2003; Jakobsen et al., 2008), since *Schizochytrium* sp. produced DHA only through anaerobic pathway of polyketide synthases (Hauvermale et al., 2006; Metz et al., 2001). Thus, various fermentation strategies were performed by controlling DO for the optimization of DHA production, e.g., stepwise DO with high DO for first cell growth stage and low DO for second lipid accumulation stage (Bailey et al., 2003; Chi et al., 2009b), or an intermittent oxygen feeding method to maintain a 50% DO level (Huang et al., 2012). The concentration of DO in a suspension of aerobic microorganism depends on the rate of oxygen transfer from the gas phase to the liquid, on the rate at which oxygen is transported into the cells (where it is consumed), and on the oxygen uptake rate (OUR) by the microorganism for growth, maintenance and production (Garcia-Ochoa and Gomez, 2009). In some aerobic cultures, oxygen demand is so high that the DO

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concentration decreases until it approaches zero, and does not increase even when the culture reaches the stationary phase, in spite of improvement in oxygen transfer rate (OTR) by increasing gas flow rate, stirring speed or the oxygen concentration in gas phase (Garcia-Ochoa et al., 2010). Furthermore, DO variance could hardly reflect the oxygen supply capacity when fermentation was conducted under DO-limited conditions (Garcia-Ochoa et al., 2000). This phenomenon evidently happened in HCDC of DHA by *Schizochytrium* sp. S31 (Chang et al., 2013a). Hence, DO is not the effective on-line parameter to optimize DHA production for scale-up of HCDC with fed batch strategy.

Oxygen transfer is often the rate-limiting step in the aerobic bioprocess due to the low solubility of oxygen in the medium. In order to supply oxygen to the culture at a non-limiting rate and to facilitate scale-up, it is therefore essential to study OUR and k_La during a bioprocess. OUR is one of the fundamental physiological parameters of culture growth (Garcia-Ochoa et al., 2010), which has been employed as on-line parameter to optimize the production of vitamin B12 or erythromycin (Wang et al., 2010; Zou et al., 2009). OUR is reciprocally influenced by OTR, especially when the fermentation occurs under DO-limited conditions. The OTR can be described as proportional to the oxygen concentration gradient, being the volumetric mass transfer coefficient, k_La the proportionality constant. The maximum OTR can be definitively determined by k_La since the maximum value of oxygen concentration gradient is limited. The k_La values are affected by many factors, such as physical properties of gas and liquid, operation conditions, geometrical parameters of the bioreactor (Garcia-Ochoa et al., 2010). Optimizing DHA production based on controlling k_La in the culture of *Schizochytrium* sp. has been previously reported (Chang et al., 2013a; Qu et al., 2010; Qu et al., 2013). However, in these studies, when the aeration rate and agitation speed was fixed, k_La was regarded as a constant parameter without considering the effect of biological oxygen consumption. In fact, k_La varies with the change of rheological properties of culture fluids during fermentation (Garcia-Ochoa and Gomez, 2009).

As already indicated, fluid viscosity (μ) appears to be related to mixing and mass transfer, including the Reynolds number and correlation for k_La (Petersen et al., 2008). Rheological properties of some microalgae cultures have been previously investigated which usually display non-Newtonian behavior (Chen et al., 1997; Geresh et al., 2002; Wileman et al., 2012). In high cell density cultures, fermentation broths become viscous which can adversely affect hydrodynamics, heat and mass transfer performance of bioreactors as well as kinetics of cell growth and product formation (Chen et al., 1997). However, for HCDC of *Schizochytrium* sp., the relationship of OUR and k_La with rheological properties has been still unknown.

In the present work, three independent cultures by fed batch strategy under three different agitation speeds (300 rpm, 450 rpm, and 600 rpm) were implemented with *Schizochytrium* sp. S31 on glycerol in 50 L bioreactor. The effects of three different oxygen supply levels on DHA production, OUR, k_La and rheological properties were studied. The aim of the present work was to propose the optimal on-line parameter rather than DO to monitor oxygen supply capacity which would be helpful to scale-up of DHA production.

2. Methods

2.1. Microorganism

The strain *Schizochytrium* sp. S31 was obtained from the American Type culture collection (ATCC) and maintained in ATCC790 By+ medium (5 g/L glucose, 1.0 g/L yeast extract and 1.0 g/L peptone in artificial seawater).

2.2. Fed-batch cultures

The seed medium was composed of glucose 100 g/L, yeast extract 6 g/L in artificial seawater. Seed culture was carried out in 7.5 L NBS Bioflo 110 fermentor (USA) containing 5 L volume with the aeration rate at 3.0 L/min, agitation speed at 600 rpm, temperature at 28 °C for 44 h. 5 L seed culture was then inoculated into the 50 L bioreactor (Shanghai Guoqiang, China).The fermentation medium contained pure glycerol 85 g/L and yeast extract 50 g/L which was described in our previous work (Chang et al. 2013a). The continuous feedback control strategy with 70% glycerol solution was employed to maintain the residual glycerol concentration at 5–20 g/L.

During the fermentation process, pH was maintained at 6.8 with H_3PO_4 (2 M) and NH_4OH (28%, w/v) automatically. The cultivation temperature was kept at 28 °C. The aeration rate and pressure was fixed at 1.26 m^3/h and 0.05 MP. DO was monitored using a polarographic electrode and was expressed as percentage of O_2 saturation. Additionally, the bioreactor was equipped with the on-line gas analysis device through which the oxygen and carbon dioxide concentration of the inlet and exhaust were determined. Consequently, the values of OUR, CO_2 evolution rate (CER) and k_La could be on-line monitored through real-time data collection software for analysis (Zhang et al., 2004).

The fermentation time was conducted for 120 h. 10 mL of fermentation broth was taken every 4 h for analysis of residual glycerol concentration, ammonia nitrogen concentration; in addition, 50 mL sample was taken every 12 h to determine dry cell weight, total lipids and fatty acid compositions.

To investigate the effect of oxygen supply levels on DHA production, OUR, CER, k_La and rheological properties of cultures, three independent cultures were experimented at the agitation speeds of 300 rpm, 450 rpm, and 600 rpm respectively. Duplicate implements for each experiment were carried out.

2.3. Determination of biomass, lipid, DHA, glycerol and nitrogen

Dry biomass, total lipid content and fatty acid compositions were determined as our previous study (Chang et al., 2013a). Glycerol was measured as described in AOCS official method Ea 6-94 (Determination of crude glycerin, titrimetric method). Nitrogen source concentration denoted as ammonium nitrogen content was determined by formol number.

2.4. Determination of OUR, CER and k_La

OUR and CER were determined based on gas analysis which were calculated from the following balance Eqs. (1) and (2) (Wang et al., 2010).

$$\mathrm{OUR}=\frac{F_{\mathrm{in}}}{V}\left[C_{\mathrm{O_2in}}-\frac{C_{\mathrm{inert\,in}}\cdot C_{\mathrm{O_2in}}}{1-(C_{\mathrm{O_2out}}+C_{\mathrm{O_2out}})}\right]\cdot\frac{273}{273+t_{\mathrm{in}}}\cdot P_{\mathrm{in}}\cdot\frac{1}{1+h}\times 10^{-5} \quad (1)$$

$$\mathrm{CER}=\frac{F_{\mathrm{in}}}{V}\left[\frac{C_{\mathrm{inert\,in}}\cdot C_{\mathrm{CO_2out}}}{1-(C_{\mathrm{O_2out}}+C_{\mathrm{CO_2out}})}-C_{\mathrm{CO_2in}}\right]\cdot\frac{273}{273+t_{\mathrm{in}}}\cdot P_{\mathrm{in}}\cdot\frac{1}{1+h}\times 10^{-5} \quad (2)$$

where F_{in} is the aeration rate, V is broth volume, $C_{inert\ in}/CO_{2\ in}/CCO_{2\ in}$ are input mass fraction of nitrogen, oxygen and carbon dioxide, P_{in} is the intensity of pressure of input gas, t_{in} is the temperature of input gas, h is the humidity of input gas.

In fermentation systems, the overall volumetric oxygen mass transfer coefficient (K_La) can be determined by measuring the total airflow and the oxygen concentration difference between the inflow and outflow air and dissolved oxygen concentration, C_L. K_La