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Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in *Scenedesmus obliquus*

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HIGHLIGHTS

- pH 7 and 27.5 °C are the optima for TAG accumulation in *Scenedesmus obliquus.*
- Lower incident light intensities result in higher yields on light.
- Highest time-averaged yields are achieved before TAG accumulation is complete.
- Highest yield achieved is 0.263 g fatty acids per mol of photons.

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ABSTRACT

Microalgae-derived lipids in the form of triacylglycerols (TAGs) are considered an alternative resource for the production of biofuels and food commodities. Large scale production of microalgal TAGs is currently uneconomical. The cost price could be reduced by improving the areal and volumetric TAG productivity. The economic value could be increased by enhancing the TAG quality. To improve these characteristics, the impact of light intensity, and the combined impact of pH and temperature on TAG accumulation were studied for *Scenedesmus obliquus* UTEX 393 under nitrogen starved conditions. The maximum TAG content was independent of light intensity, but varied between 18% and 40% of dry weight for different combinations of pH and temperature. The highest yield of fatty acids on light (0.263 g/mol photon) was achieved at the lowest light intensity, pH 7 and 27.5 °C.

1. Introduction

Microalgae are considered one of the most promising future feedstocks for sustainable supply of commodities for both food and non-food products (Draaisma et al., 2012; Wijffels and Barbosa, 2010). Lipids derived from microalgae in the form of triacylglycerol (TAG) can for example be used for biodiesel production (Chisti, 2007) and certain microalgal oils might in part substitute functionalities of major vegetable oils in food applications (Draaisma et al., 2012).

Many microalgae species have the ability to produce TAG. Under optimal growth conditions they produce very low quantities of TAG, but when exposed to nitrogen starvation TAG accumulation is induced and contents as high as 40% of dry weight can be reached (Breuer et al., 2012). When exposed to nitrogen limitation, the production rate of functional biomass (i.e. protein, DNA, RNA, chlorophyll) is impaired. The difference between the rate at which electrons are generated by photosynthesis and the lower rate at which these electrons can be used to produce functional biomass can at least partially be used to produce TAG. TAG functions as a storage component for energy and carbon, but in addition its formation also prevents photo-oxidative damage to the cell by incorporating excess photosynthetically derived electrons (Hu et al., 2008).

Costs of large scale production of microalgae-derived TAGs currently exceed those for the production of vegetable oils (Norsker et al., 2011; Ratledge and Cohen, 2008). The cost price could be reduced by improving the areal and volumetric productivity of TAG. Furthermore, the economic value could be enhanced by improving the TAG quality, which is determined by its fatty acid composition. TAG content and quality vary between microalgae species and depend on cultivation conditions (Breuer et al., 2012; Griffiths et al., 2011). Selection of a suitable species and optimization of cultivation conditions is therefore of paramount importance.

In previous research, nine microalgae species were selected as most promising for TAG production from a literature survey among

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96 microalgae strains. These nine strains were experimentally evaluated and *Scenedesmus obliquus* UTEX 393 was identified as the most promising strain for TAG production (Breuer et al., 2012).

Light intensity, pH, and temperature are important cultivation parameters for microalgal growth and could therefore affect TAG productivity, yield and quality (Hu et al., 2008). Many investigations focussed on the impact of these process conditions on nitrogen replete growth whereas only little information is available about the impact of these process conditions on TAG accumulation induced by nitrogen starvation. For example, the impact of light intensity on the photosynthetic rate and photosynthetic efficiency under light limited growth is investigated extensively for many different microalgal species (Janssen et al., 2000; Jassby and Platt, 1976; MacIntyre et al., 2002). Also, the impact of cultivation conditions on the nutritional value of marine microalgae for aquaculture applications, such as temperature (Renaud et al., 2002, 1995) or light intensity (Guedes et al., 2010), has been the topic of many publications. Finally, many investigations focussed on determining cultivation conditions that result in maximum growth rates. For example, optimal conditions for nitrogen replete growth of *S. obliquus* are pH 7 (Hodaifa et al., 2010a) and a temperature of 25–30 °C (Hodaifa et al., 2010b; Xin et al., 2011).

These investigations did not aim at maximizing TAG content or TAG productivity and neither did these cultivation conditions contribute to high TAG contents or productivities. TAG accumulation is most commonly achieved by applying nitrogen starvation. Optimal cultivation conditions for TAG accumulation under these nitrogen deplete conditions may be very different from those for growth under nitrogen replete conditions.

Knowledge about this topic is limited. Most knowledge is available about the impact of light intensity on TAG accumulation under nitrogen starved conditions, but a clear consensus is lacking. For example, both observations that lipid content is affected only to a minor extent by light intensity (Pal et al., 2011; Simionato et al., 2011) as well as observations that lipid content increases with light intensity have been made (Liu et al., 2012). Too few results are available to draw conclusions about the exact impact of pH and temperature on TAG accumulation under nitrogen starved conditions. However, it has been reported that high pH values can contribute to TAG accumulation (Gardner et al., 2010; Guckert and Cooksey, 1990; Santos et al., 2012).

In addition to the limited availability of knowledge on this topic, reported results are sometimes difficult to interpret because the impact of nitrogen starvation on TAG accumulation is not always completely isolated from the impact of the other investigated cultivation conditions. For example, in some investigations lipid accumulation was only evaluated at one arbitrarily chosen time point, while the investigated cultivation condition affected the moment at which the culture became nitrogen starved, and thus varying the duration of nitrogen starvation. This may lead to erroneous interpretation of the results and the outcomes might have been different when a different time point would have been chosen. It

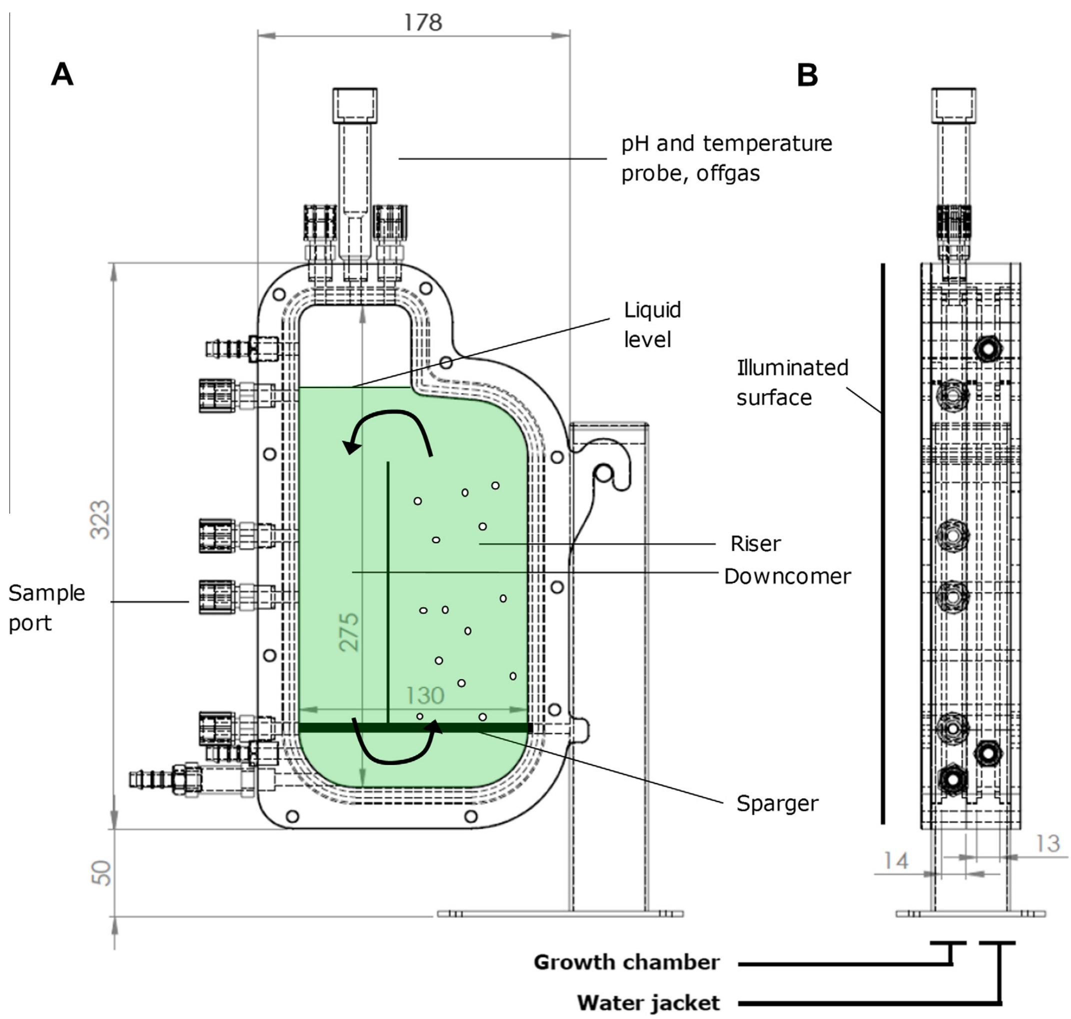

Fig. 1. Design of the bioreactor used. The water jacket was connected to an external temperature-controlled water bath. Illumination was provided from the side of the growth chamber (left side of Fig. B). The depth of the growth chamber was 14 mm and the working volume in the growth chamber was 380 ml.