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Biofuels from microalgae: Photoconversion efficiency during lipid accumulation

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HIGHLIGHTS

- Photoconversion efficiency during lipid accumulation was determined.
- The enthalpy of combustion of microalgal lipids and biomass was measured.
- Nitrate availability influences volumetric lipid productivity and efficiency.
- Specific rates of lipid accumulation show saturation at high light intensities.

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ABSTRACT

The accumulation of storage lipids in oleaginous microalgae can be induced by a targeted nutrient limitation. Experiments with varying concentrations of nitrate in the culture medium showed differing volumetric productivities of *Phaeodactylum tricornutum* in batch experiments. This was partially attributable to the differentiated ability of cultures to absorb light. Apart from that, it was demonstrated that storage molecule accumulation follows kinetics that show saturation at high photon flux densities. The measurement of the photoconversion efficiency (PCE) based on a rigorous balancing of absorbed light energy and changes in the enthalpy of combustion of biomass during nutrient depletion. In batch experiments the PCE was increased more than twofold, from 2.48% at low nitrate concentrations to a maximum value of 5.65%, by increase of the nitrogen availability.

1. Introduction

A wide range of comprehensive studies have been published on the feasibility of microalgae as feedstock for biofuel production (e.g. Chisti, 2008; Greenwell et al., 2010; Tredici, 2010).

Microalgae cultivation is not restricted to arable land (Hu et al., 2008). Moreover, the fresh water demand of production in closed photobioreactors is substantially lower than for conventional farming of oil crop plants and especially low when water recycling is implemented or algae are cultivated in wastewater (Yang et al., 2011). Moreover, conventional oil crop plants, e.g., rapeseed or sunflower, contain a maximum lipid content of only 5% relating to the entire plant (Amaro et al., 2011). By comparison, some algal strains can exhibit high oil contents of more than 80% of their dry mass and show high areal productivities (Spoehr and Milner, 1948; Spolaore et al., 2006; Tredici, 2010). In general, a better utilization of sunlight is expected for oil production with microalgae than with higher plants (Chisti, 2007).

A positive net energy balance and economic feasibility are prerequisites for any large scale phototrophic production of microalgal oil. Both demand a high efficiency with which solar light impinging on the given ground area is converted into biomass, a key performance indicator usually denoted as photoconversion efficiency (PCE). The thermodynamic limit of PCE is estimated to be 12.4% of incident solar energy. It is further diminished to ca. 5% due to losses caused by reflection, photorespiration, photosaturation and inhibition (for a comprehensive overview see e.g. Schlagermann et al., 2012; Tredici, 2010). Minimization of the latter losses is aimed at by the improvement of reactor design and set-up (Posten, 2009). Its optimization potential is demonstrated by the variability of PCE depending only on the reactor types as shown by a study that compares literature productivity data of the most commonly found reactor designs and converts them into PCE values. The resulting values amounted to 1.5% for raceway ponds, 3% for tubular reactors and 5% for vertical panels while all data are denoted as base case scenarios on an annual basis (Norsker et al., 2011).

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Based on the assumption that ideal growth conditions can be achieved by an ideal reactor the attainable PCE will be determined by the physiology of the respective algae strain and is subject to variability depending on the metabolism of the microalgae. Under optimal growth conditions cells primarily synthesize polar lipids, such as membrane phospholipids. Some strains respond to stress conditions, e.g. photo-oxidative stress or high salinities, with the accumulation of neutral lipids. The latter consist mainly of triacylglycerides (Hu et al., 2008). Targeted limitation of nutrients in the culture medium, such as the nitrogen or phosphorous source, has been demonstrated to be the most efficient method to induce storage lipid accumulation (Spoehr and Milner, 1948). However, PCE is assumed to be lower during oil accumulation compared to optimal growth conditions. Firstly, this results from reduced growth rates under stress conditions (Schlagermann et al., 2012; Wijffels and Barbosa, 2010). Secondly, the synthesis of lipids requires additional ATP and NADPH as well as further enzymatic steps compared to the conversion of photosynthetically fixed carbon to e.g. carbohydrates. Despite the higher enthalpy of combustion these longer metabolic pathways of synthesis and remobilization of lipids are expected to reduce their efficiency as storage molecules. Impact on the energy balance is further aggravated because lipids are subject to turnover, even though this effect is not quantified yet (Wilhelm and Jakob, 2011).

Several scenario studies resort to the critical parameter PCE to assess the potential as well as areal requirements of microalgal biofuel production (Schlagermann et al., 2012; Wijffels and Barbosa, 2010). However, the actual photosynthetic efficiency of microalgal oil accumulation has not been quantitatively determined, yet. Besides, the PCE reacts sensitively to changes in the enthalpy of combustion of biomass. The evaluation of PCE and prediction of productivity is usually addressed on the basis of a representative biomass composition and its corresponding enthalpy of combustion (e.g. Norsker et al., 2011). This approach is reasonable for scenario studies and for estimation of growth efficiency under nutrient replete conditions. However, nutrient limitation and the concomitant storage lipid accumulation cause significant changes of the enthalpy of combustion which needs to be taken into account for accurate measurements of the PCE during this growth phase.

The focus of the experimental work presented in this paper was the quantification of PCE during storage lipid accumulation. Moreover, a clear distinction of the effects of a nutrient limitation on lipid productivity at the reactor level and at the physiological level should provide further insights into the kinetics of product formation. Being a potential source of biodiesel, *Phaeodactylum tricornutum* was selected as model organism. Besides, this species exhibits a high amount of additionally marketable polyunsaturated fatty acids, such as eicosapentaenoic acid (Reitan et al., 1994; Yongmanitchai and Ward, 1991) and its genome is fully sequenced (Bowler et al., 2008), which makes the organism popular for research in photobiotechnology.

2. Methods

2.1. Strain and culture conditions

P. tricornutum, strain 1090-1a, was obtained from Culture Collection of Algae (*SAG*), University of Göttingen, Germany. The inoculum for the experiments were cultivated in shaking flask cultures incubated at 21 °C for 3 weeks with LED illumination adjusted to 150 μmol/(m^2 s). The culture medium was based on a medium published by Mann and Myers (1968) with the following modifications: NaCl concentration was 27.0 g/L, K_2HPO_4 concentration was 0.15 g/L and $MgSO_4{\cdot}7H_2O$ was reduced to a final concentration of 0.6 g/L. After 9 days of growth 5 ml of a solution of 35 g/L K_2HPO_4 and 144 g/L $MgSO_4{\cdot}7H_2O$ were added to the cultures with an initial $NaNO_3$ concentration higher than 1.5 g/L in order to avoid phosphorous or sulfur limitation. Tris buffer was omitted to prevent growth of heterotrophic organisms (Fábregas et al., 1993). Concentrations of $NaNO_3$ were varied in the experiments but adjusted to 1.0 g/L for the preculture. Besides, 30 mg/L $Na_2SiO_3{\cdot}5H_2O$ were added.

To account for slight differences in the optical density of cultures used for inoculation, the volume of the inoculum was adjusted to attain equal starting conditions for the respective experiments.

All chemicals, purchased from Carl Roth, were of analytical grade (p.a.). In all experiments pH was kept constant at pH 7.7 by controlled addition of CO_2 during cultivation.

2.2. Midiplate photobioreactor

Midiplate reactors were developed at the Institute of Bioprocess Engineering at Karlsruhe Institute of Technology (KIT). They consisted of stainless steel frames with glass slides attached with silicone in grooves of the side panels (Fig. 1). Removable upper and lower sections of the frame were sealed with 2 mm silicone gaskets and were provided with apertures where pH sensor (Polylite Plus Arc 225, Hamilton), needles for sampling and inoculation as well as tubes for exhaust gas analysis could be connected. The cultivation compartment with dimensions of 350 × 200 × 20 mm (height × width × depth) was filled with 1.2 L culture medium. The temperature was measured with PT100 resistance thermometers and cooling of reactors was achieved by controlled flow of cooling water in hollow side panels of the steel frame. Temperature

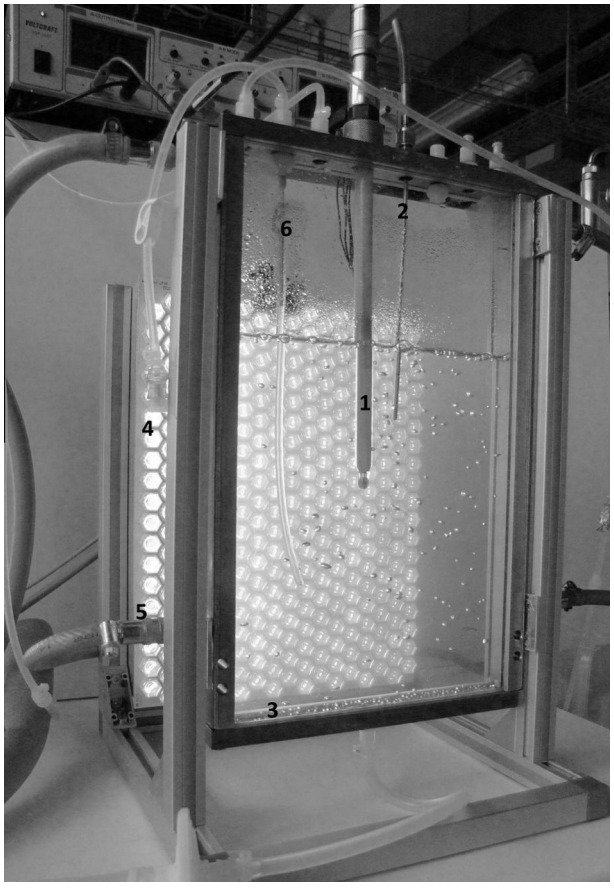

Fig. 1. Midiplate reactor. (1) Polylite plus pH sensor, (2) PT100 resistance thermometer, (3) polytetrafluoroethylene membrane, (4) LED panel with collimating lenses, (5) connection for cooling water, and (6) sampling tube.