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Enhancement of fatty acid production of *Chlorella* sp. (Chlorophyceae) by addition of glucose and sodium thiosulphate to culture medium

Fu-Ying Feng, Wen Yang, Gui-Zhen Jiang, Yi-Nong Xu*, Ting-Yun Kuang

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China

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Abstract

The effects of glucose, sodium thiosulphate and a combination of these two compounds in culture medium on growth kinetics and fatty acid production of *Chlorella* sp. were investigated. Two different concentrations (2.5 mmol and 5.0 mmol) of both components in culture medium were used. Results showed that glucose induced an increase in cell concentration and sodium thiosulphate enhanced accumulation of fatty acids in the cells. When cells were grown in a medium containing both glucose and sodium thiosulphate, the production of fatty acids depended on the concentration of these two components. At a given glucose concentration, an increase in fatty acid production was obtained by increasing the sodium thiosulphate concentration. In contrast, an increase in glucose concentration caused a decrease in fatty acid production when the sodium thiosulphate concentration was fixed. These results suggest that an appropriate concentration of glucose in combination with sodium thiosulphate can enhance the accumulation of lipids of *Chlorella* sp. cells.

Keywords: *Chlorella* sp.; Fatty acids; Glucose; Mixotrophic; Sodium thiosulphate

1. Introduction

Marine microalgae are primary producers and play an important role in marine ecosystems. They have high potential commercially, because they are a major source of the long-chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenonic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) [1,2]. Marine algae generally have a low growth rate and biomass under natural condition. Fortunately, some microalgae can grow in a mixotrophic way by digestion of exogenous carbon. In the presence of exogenous carbon, the growth rate and biomass and PUFAs of these algae were enhanced [3]. Many efforts are being made to enhance the production of PUFAs by culturing marine microalgae with exogenous carbon [4]. Glucose is most commonly used as an exogenous carbon in microalgae culture to enhance lipid production [5]. However, in the process of degradation of glucose, plant cells may produce reactive oxygen (e.g. $^1O_2$ and O_2^-), which can heavily damage bio-macromolecules [6,7]. Membranes and membrane proteins are most easily attacked by reactive oxygen. Photosynthesis inevitably produces reactive oxygen, but plants have elaborate systems to reduce the level of reactive oxygen [8]. Addition of glucose, however, probably produces more reactive oxygen than microalgae cells themselves can scavenge. Sodium thiosulphate, as a reducing reagent, could scavenge reactive oxygen effectively and protect cells against the damage of reactive oxygen produced by biodegradation of exogenous organic carbon and it is used for this purpose in cultures of bacteria and zooblasts [9]. We previously reported that the cyanobacterium *Synechocystis* sp. PCC 6803 transformed to heterotrophic growth from autotrophy under high concentrations of glucose, which suffered the degradation of membrane lipids. A protective effect of sodium thiosulphate on membrane lipid degradation induced by glucose was observed [6].

Marine *Chlorella* sp. is a significant potential source of EPA and is widely used in aquaculture in China. In this work, the effects of glucose and sodium thiosulphate on growth and fatty acid production of marine *Chlorella* sp. were investigated.

* Corresponding author.
E-mail address: yinongxu@ns.ibcas.ac.cn (Y.-N. Xu).

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2. Materials and methods

2.1. Microalgae and culture conditions

The marine microalga *Chlorella* sp. was gifted from the Institute of Oceanology, Chinese Academy of Sciences. For inoculation, 1 ml culture in the exponential growth phase was transferred into 50 ml artificial seawater [10], which contained initially different concentration of sodium thiosulphate and glucose, as described in Table 1. Cells were grown at 22 °C and with continuous light (70 μE m^{-2} s^{-1}) by shaking at 135 rpm on an orbital shaker in a culture chamber (Cabinet Model RXZ 300B; Jiangnan Instrument Factory, Ningbo, China).

2.2. Measurement of Chlorella sp. growth kinetics

To determine the cell density and growth status, the absorbance at 750 nm was measured every other day during growth with a spectrometer as described by Cerón-García et al. [3].

2.3. Lipid extraction and separation

After 15 days of growth (cells at the stationary phase), the cells in 40 ml culture medium were harvested by centrifugation of 10 min at 8000 × *g*. Lipids were extracted according to the method of Bligh and Dyer [11] and kept in 2 ml of methanol/chloroform (1:1, v/v). Polar and non-polar lipids were separated by single dimension TLC on precoated silica gel (type of G, 10 cm × 10 cm, manufactured by Qingdao Ocean Chemical Co. Ltd.) using a solvent system (hexane/diethyl ether/acetic acid, 80:20:1, v/v) according to the method described by Dahlqvist et al. [12]. Lipid bands on TLC plate were visualized under UV light (366 nm) after spraying the plate with 0.01% (w/v) primulin in the mixture of acetone/water (60:40, v/v) [13].

2.4. Quantitative analysis of fatty acids

To determine the fatty acid production, 50 μl of total extracts was transmethylated with 5% H_2SO_4 in methanol at 85 °C for 1 h. Heptadecanoic acid was added as an internal standard. Fatty acid esters were analyzed on a HP6890 plus gas chromatograph, equipped with a Hewlett Packard high performance capillary column (HP-INNOWax, 30 m × 0.25 mm, i.d.). N_2 was used as carrier gas at the rate of 0.8 ml/min. The oven temperature was programmed as follows: the initial 3 min held at 170 °C, the 4th to the 11th min ranged linearly from 170 °C to 210 °C and the following 11 min held at 210 °C, the last 10 min linearly increased from 210 °C to 230 °C. The temperature of the flame ionization detector was 230 °C. The separate components were identified by retention time with reference to known standards purchased from *Supelco* (lot number: LA-98232).

Table 1
Various initial concentrations of sodium thiosulphate and glucose and corresponding symbol

Symbol	Sodium thiosulfate (S) (mM)	Glucose (G) (mM)
S0.0G0.0	0.0	0.0
S0.0G2.5	0.0	2.5
S0.0G5.0	0.0	5.0
S2.5G0.0	2.5	0.0
S2.5G2.5	2.5	2.5
S2.5G5.0	2.5	5.0
S5.0G0.0	5.0	0.0
S5.0G2.5	5.0	2.5
S5.0G5.0	5.0	5.0

3. Results and discussions

3.1. Effect of glucose and sodium thiosulphate on the growth kinetics of marine Chlorella sp.

Cell density of *Chlorella* sp. was estimated by measuring turbidity at 750 nm with a spectrophotometer. To ensure that the turbidity represented well the cell density, a relationship between turbidity and cell density was established. *Chlorella* sp. cells grown in control medium to the stationary phase were concentrated threefold and then gradually diluted before the turbidity of cells was measured. As shown in Fig. 1, the value of turbidity (as absorbance at 750 nm) within the range of 0.028–1.384 was linearly correlated with the cell density.

The effects of glucose, sodium thiosulphate and the combination of these two compounds on the growth kinetics of *Chlorella* sp. are summarized in Fig. 2. Under all growth conditions, *Chlorella* sp. cells growth kinetics displayed a typical growth S-curve, i.e. there were a lag phase from 0 to 3rd day, an exponential phase from 4th to 13th day and a stationary phase after 13 days of growth. Initial addition of glucose to the culture medium resulted in an increase in cell

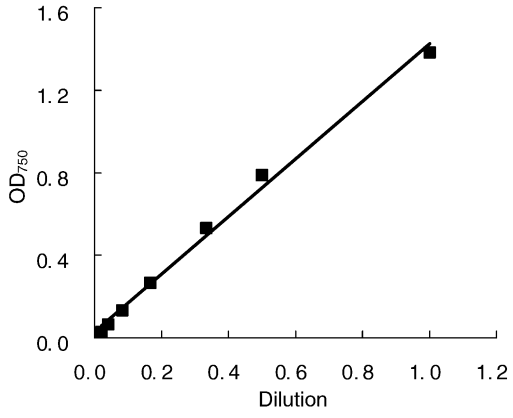

Fig. 1. Relationship between the value of turbidity (as absorbance at 750 nm) and cell density of marine *Chlorella* sp. (see text for details).