J. Microbiol. Biotechnol. (1998), **8**(4), 409 – 411

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

NOTE

Enhanced Cell Growth of *Chlorella* sp. KR-1 by the Addition of Iron and EDTA

SUNG, KI-DON, JIN-SUK LEE*, CHUL-SEUNG SHIN, AND SOON-CHUL PARK

Biomass Team, KIER, P.O. Box 5, Taedok Science Town, Taejon 305-340, Korea

Received: February 25, 1998

Abstract The effects of iron and EDTA on the growth of *Chlorella* sp. KR-1, a highly CO_2 tolerant fresh water microalga, have been determined. The algal growth was significantly affected not only by iron concentrations in the medium but by the ratio of iron to EDTA. The linear growth rate and the final cell concentration are increased with the supplementation of EDTA. Enhanced growth of *Chlorella* sp. KR-1 by the supplementation of EDTA was mainly due to the fact that the supply of iron to the algal culture had been possible for a longer time. When *Chlorella* sp. KR-1 is cultured in the medium of iron-15H-EDTA, the linear growth rate and the final cell concentration are at their maximum, 0.88 g/l·day and 9.1 g/l, respectively. The results show that *Chlorella* sp. KR-1 may be used for mass cultivation to fix CO_2 from stack gases.

Key words: CO_2 fixation, *Chlorella* sp. KR-1, iron, EDTA

Biological CO_2 fixation using microalgae is a promising approach to reducing the global warming [2, 4, 7]. It has been suggested that the direct fixation of discharged gases from industrial sources reduces the cost of pretreatment, but imposes extreme conditions on microalgae such as high concentrations of CO_2 and the presence of inhibitory compounds. The best suited microalgal strain for this purpose should be selected. Some work has been carried out to isolate a highly CO_2 tolerant algal strain [1, 3, 6-8]. Most work has been focused on determining the cultural characteristics of the isolated strain under various environmental conditions such as pH, temperature, CO_2 concentrations in feeding air, and aeration rates [1, 3, 5, 7, 8].

Some minor components in the culturing medium play a key role in the algal cell growth. The importance of iron for the growth of algae is well substantiated; it is a key element in metabolism, being a constituent of cytochrome, and is known to affect the synthesis of chlorophyll, a key component for photosynthesis. Since the iron, added in the form of inorganic salts, is easily precipitated, a long-term supply is critical. Now, iron is mostly supplied in the form of chelated complexes, preferably bound to EDTA. Little work has been done to determine the effects of iron on the CO_2 fixation by microalgae [10]. Yanagi *et al.* reported that the growth limiting factors were minor elements like iron, zinc, and copper [10]. Among them, iron was the most important [10]. Since every algal strain has different cultural characteristics, the suitable iron and EDTA concentrations should be different.

Recently, Sung *et al.* [6] isolated an alga, named *Chlorella* sp. KR-1, which has good tolerance to high concentrations of CO_2. The strain showed good growth at high CO_2 concentrations up to 50% CO_2. As a preliminary step for the optimization of culturing media composition, the effect of iron and EDTA concentrations on the growth of *Chlorella* sp. KR-1 was determined.

Chlorella sp. KR-1 was cultured in the medium which had the following composition: (in mg/l); KNO_3, 5000; $MgSO_4 \cdot 7H_2O$, 2500; KH_2PO_4, 1250; H_3BO_3, 2.86; $MnSO_4 \cdot 7H_2O$, 2.5; $ZnSO_4 \cdot 7H_2O$, 0.222; $CuSO_4 \cdot 5H_2O$, 0.079; Na_2MoO_4, 0.021. Iron and EDTA concentrations have been varied with the experimental conditions. The initial pH of the medium was adjusted to 5.5.

Microalgal culture experiments were conducted to determine the effect of iron and EDTA on the growth of *Chlorella* sp. KR-1. The algal strain was cultured in 200 ml medium in 400-ml glass tubes (3.5 cm × 40 cm) and growth rates were monitored with different iron and EDTA concentrations. They were illuminated by fluorescent tubes at 100 mol/m^2·sec. The seed culture was centrifuged and washed before inoculation. Samples were removed daily from the vessels to determine the algal growth and pH of the medium. The temperature of the culture media was maintained at 25°C. The pH of the

*Corresponding author
Phone: 82-42-860-3553; Fax: 82-42-860-3132;
E-mail: bmjslee@sun330.kier.re.kr

medium was not regulated. The concentration of CO_2 was controlled to 10% with a gas mass flow controller (905C-PS-BM-11, Sierra Instruments Inc., U.S.A.) and monitored by an on-line CO_2 analyzer (IR-8400, Summit Analyzers Inc., U.K.). The gas flow rate was fixed to 0.5 vvm.

The algal growth was determined by measuring the absorbance at 660 nm using a spectrophotometer (HP8452A, Hewlett-Packard Inc., U.S.A.) which was converted into dry cell weight. Light intensities were measured by a light sensor (LI-250, LI-COR Inc., U.S.A.).

To develop a suitable medium composition for *Chlorella* sp. KR-1, the effect of iron and ethylenediaminetetraacetic acid (EDTA) concentrations in the culture medium on the growth of *Chlorella* sp. KR-1 was investigated. M4N medium [9] developed for *Chlorella* sp. HA-1, which contains 3 mg $FeSO_4 \cdot 7H_2O$, was used as the basic medium for culturing the new isolate, *Chlorella* sp. KR-1. Comparative experiments were conducted using the following culture media: "iron-7", a medium which contained 7 mg $FeSO_4 \cdot 7H_2O$ instead of 3 mg in 1 l M4N medium; and "iron-7-EDTA", an iron-7 supplemented with 1 mg $Na_2EDTA \cdot 2H_2O$. The courses of growth of algae are shown in Fig. 1. As shown in the figure, the growth curve of M4N culture showed a linear ascent during the first 4 days, after which the growth rate suddenly decreased. By contrast, the curves of iron-7 and iron-7-EDTA cultures continued to ascend linearly for 5 days, after which the growth slowly decreased. The net increase in algal quantity attained at the end of the experiment was about 2 twice as large in the iron-7-EDTA culture as in the M4N culture. The probable cause of the decline of the growth rate in the M4N culture was a shortage of iron. Apparently, *Chlorella* sp. KR-1, the new isolate, needs more iron than *Chlorella* HA-1. The linear growth rate in the iron-7-EDTA culture is 30% higher than that in the iron-7 culture.

Although it is well known that EDTA plays an important role in enhancing the growth of algae, no quantitative information is available for *Chlorella* sp. KR-1 or other algal cell cultures. The effect of iron/EDTA on algal growth has been determined. As shown in Fig. 2, the linear growth rate in both cultures is about 0.62 g/l-day and independent of the iron/EDTA. Iron/EDTA, however, affects the period during which the linear growth rate was maintained. While the growth curve of the iron-7-EDTA culture showed a linear ascent during the first 5 days after which the growth rate suddenly decreased, the growth curve of iron-7H-EDTA culture, EDTA-strengthened media, showed a linear ascent during 5 days, after which the growth slowly decreased. The possible cause of the decline of growth in the iron-7H-EDTA culture may be a shortage of iron.

To investigate the cause of the growth retardation, comparative experiments were carried out using a culture media having different iron concentrations and iron/EDTA was fixed at 0.2. As shown in Fig. 3, the linear growth rates were not significantly dependent on iron concentrations during the first 5 days. As was expected, the period during which the linear growth lasted,

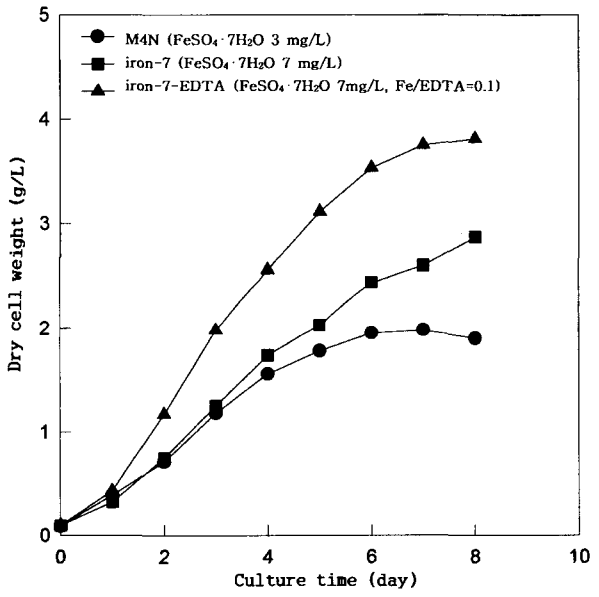

Fig. 1. Effect of iron concentrations on the growth of *Chlorella* sp. KR-1.

The cultures were illuminated at 100 μmol/m^2·sec and cultured at 25°C with air containing 10% CO_2.

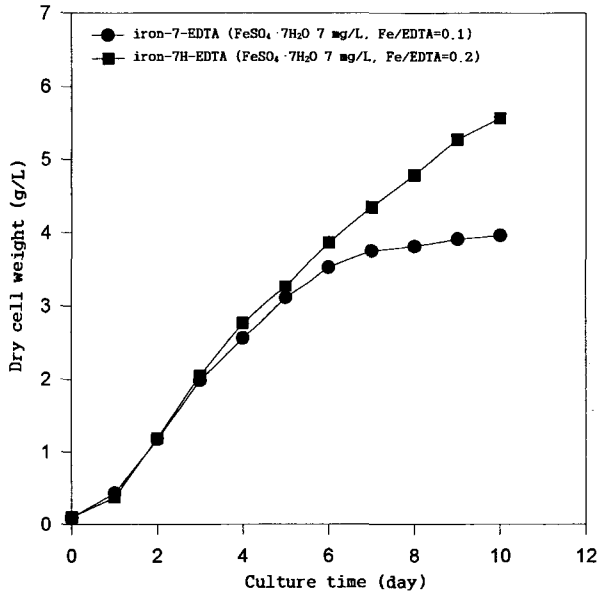

Fig. 2. Effect of the ratio of Fe to EDTA on the growth of *Chlorella* sp. KR-1.

The cultures were illuminated at 100 μmol/m^2·sec and cultured at 25°C with air containing 10% CO_2.

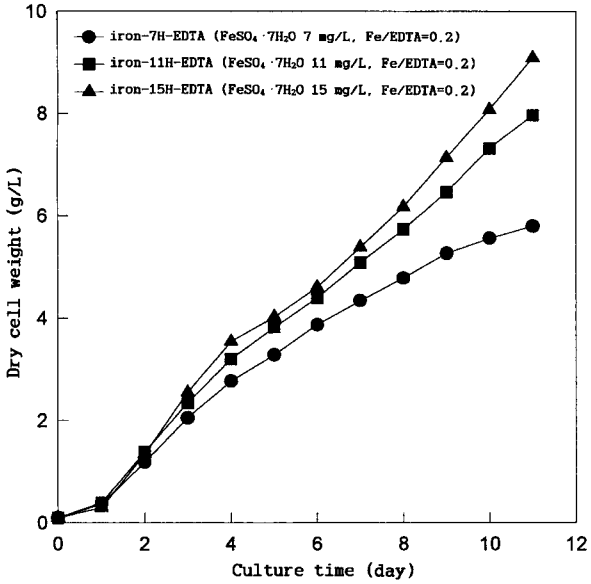

Fig. 3. Effect of iron concentrations on the growth of *Chlorella* sp. KR-1 cultured in the medium supplemented with EDTA.

The cultures were illuminated at 100 μmol/m^2·sec and cultured at 25°C with air containing 10% CO_2.

however, got longer with the increase of iron concentration in the culture. The growth curve in the iron-15H-EDTA culture showed continuous linear ascent which lasted practically indefinitely. The final cell concentration and the linear growth rate in the iron-15H-EDTA culture were 9.1 g/l and 0.88 g/l·day. Enhancements in final cell concentration and linear growth rates in *Chlorella* sp. KR-1 cultured with iron-15H-EDTA medium suggested that *Chlorella* sp. KR-1 requires a higher iron concentration than *Chlorella* sp. HA-1. Enhanced growth in the algal culture with the addition of iron was possibly due to higher rates of the nitrogen assimilation and the chlorophyll synthesis because iron had been known as a key element in metabolism. In conclusion, iron played a key role for CO_2 fixation by *Chlorella* sp. KR-1. Supplementation of EDTA was required to supply iron to the algal culture for a long time. Finally, these results indicated the feasibility of using *Chlorella* sp. KR-1 for mass cultivation to fix CO_2 in stack gases. Further work for the optimization of media composition is underway in our laboratory.

Acknowledgments

This project was supported by the Korean Ministry of Environment and Ministry of Science and Technology.

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