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SHORT COMMUNICATION

ISOLATION OF A NEW HIGHLY CO_2 TOLERANT FRESH WATER MICROALGA *CHLORELLA* SP. KR-1

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Abstract – A fresh water microalga, which has tolerance to high concentrations of CO_2, was isolated. The KR-1 strain was identified as a genus *Chlorella*. Though *Chlorella* KR-1 showed maximum growth at 10 % (v/v) CO_2, the strain showed a good growth rate up to 50 % (v/v) CO_2. The results indicated the feasibility of the KR-1 strain for massive cultivation using condensed stack gases.

Key words : *Chlorella* sp., Highly CO_2-Tolerant Microalga, CO_2 Fixation

INTRODUCTION

Direct use of flue gas reduces the cost of pretreatment but imposes extreme conditions on microalgae such as high concentrations of CO_2 and the presence of inhibitory compounds like NO_x and SO_x. Most microalgal strains are known to be critically inhibited with air containing 50 ppm SO_x [Watanabe et al., 1992; Kurano et al., 1995]. Therefore, direct CO_2 fixation by culturing microalgae may be possible only for the flue gas from LNG-fueled plants which do not contain SO_x.

The flue gas from other fuels containing sulfur, like coal and heavy oil, should contain a significant amount of SO_x. It is suggested that the flue gas should be condensed to remove the inhibitory compounds by physicochemical methods such as absorption, adsorption, or membrane separation and then purged into the microalgal culture to fix CO_2 [Kodama et al., 1993; Yun and Park, 1997]. If the flue gas is once condensed by chemical and/or physical means, the gas is going to be purified as CO_2. Therefore the gas fed for microalgal cultivation could have up to 100 % CO_2 and a trace of toxic compounds. It has been reported, however, that concentrations of CO_2 above 5 % inhibit the growth of microalgae [Silva and Pirt, 1984; Lee and Tay, 1991]. Strains that grew fast at as high CO_2 as possible would be required.

Although a few works have been recently reported on the isolation of highly CO_2-tolerant microalgae [Watanabe et al., 1992; Kodama et al., 1993; Hanagata et al., 1992; Takeuchi et al., 1992], only Hanagata et al. were successful in isolating fresh water microalgae which grew fast up to 50 % CO_2 [Hanagata et al., 1992]. The CO_2-tolerant microalgae were identified to be *Chlorella* and *Scenedesmus* sp.

The objective of this work was to isolate new microalgae which had a high growth rate in the culture at high CO_2 concentrations and to determine their CO_2 tolerances.

MATERIALS AND METHODS

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1. Isolation of Microalgae

Microalgae were isolated from water samples in the vicinity of a Korean thermal power plant, Youngwol, by the methods described by Watanabe [Watanabe et al., 1992].

2. Culture Conditions

Microalgal culture experiments were conducted to determine the culture characteristics of the isolate, named the KR-1 strain. The algal strain was cultured in 200 ml modified M 4N medium [Watanabe et al., 1992] in 400 ml glass tubes, and growth rates were monitored at different CO_2 concentrations. They were illuminated at a light intensity of 110 μmol/m^2-sec by fluorescent tubes. 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 30 °C unless otherwise indicated. The initial pH of the medium was 5.5 and was not regulated. The concentration of CO_2 was regulated by controlling the flow rates of air and CO_2 with a gas mass flow controller (905C-PS-BM-11, Sierra Instruments Inc., Monterey, USA) and measured by an online CO_2 monitor (IR-8400, Summit Analyzers Inc., Livermore, USA). The gas flow rate was fixed at 0.5 vvm.

3. Assay

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

RESULTS AND DISCUSSION

1. Isolation of Microalgae

A new fresh water microalga was isolated which could grow well at 50 % CO_2. It is a single-cell green alga (named to strain KR-1), and the cell size is about 3-5 μm (Refer to Fig. 1). The KR-1 strain was identified as a genus *Chlorella*.

2. Effects of CO_2 Concentrations

Chlorella KR-1 was cultured under continuous illumination with different concentrations of CO_2 ranging from air-level to

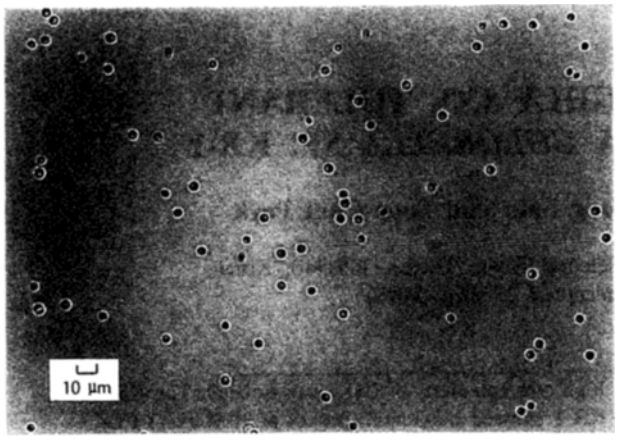

Fig. 1. Micrograph of *Chlorella* sp. KR-1.

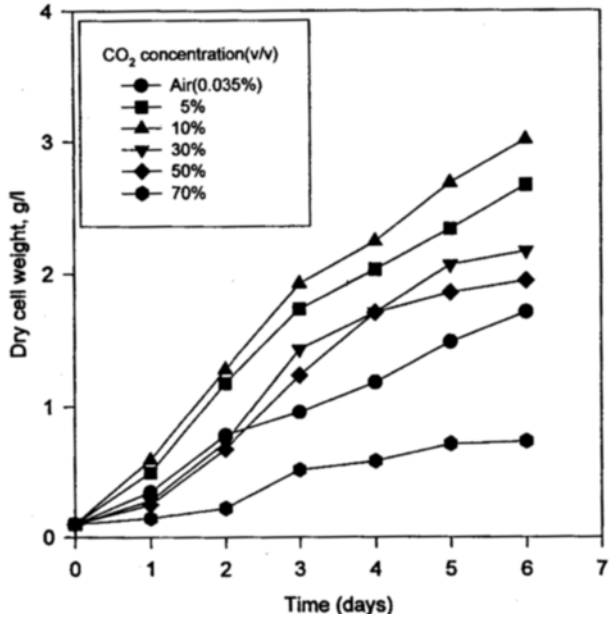

Fig. 2. Growth of *Chlorella* KR-1 at different concentrations of CO_2. The cultures were grown at 25 °C and a light intensity of 110 μmol/m²-sec. pH in the medium was 4.1 at an initial stage.

70 % CO_2. Growth characteristics were evaluated by the following two parameters: the linear growth rate and the maximum cell concentration. Fig. 2 shows the growth of the KR-1 strain at different CO_2 concentrations.

The optimum growth rate was found in the culture at 10 % CO_2. The linear growth rate of KR-1 cultured with 10 % CO_2 was determined to be about 0.667 g/*l*-day and the maximum cell concentration was determined to be 3.01 g/*l* after 6 day cultivation. KR-1 maintained still high growth rates and cell concentrations at high CO_2 concentrations, of 30 % and 50 %. However, the lag period before starting growth tended to last longer at higher CO_2 concentrations. Although the growth rate is remarkably low at 70 % CO_2, *Chlorella* KR-1 still grows and reaches 0.71 g/*l* after 5 day cultivation, which was 7 times as high as that of the inoculum. All highly CO_2-tolerant microalgae ever reported [Hanagata et al., 1992; Kodama et al., 1993; Takeuchi et al., 1992] have no CO_2-fixation activity or growth at 70 % CO_2. These results show that *Chlorella* KR-1 is a promising strain to grow at extremely high CO_2 concentrations.

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