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Chlorella vulgaris culture as a regulator of CO_2 in a bioregenerative life support system

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Abstract

It is the primary task for a bioregenerative life support system (BLSS) to maintain the stable concentrations of CO_2 and O_2. However, these concentrations could fluctuate based on various factors, such as the imbalance between respiration/assimilation quotients of the heterotrophic and autotrophic components. They can even be out of balance through catastrophic failure of higher plants in the emergency conditions. In this study, the feasibility of using unicellular *Chlorella vulgaris* of typically rapid growth as both "compensatory system" and "regulator" to control the balance of CO_2 and O_2 was analyzed in a closed ecosystem. For this purpose, a small closed ecosystem called integrative experimental system (IES) was established in our laboratory where we have been conducting multi-biological life support system experiments (MLSSE). The IES consists of a closed integrative cultivating system (CICS) and a plate photo-bioreactor. Four volunteers participated in the study for gas exchange by periodical breathing through a tube connected with the CICS. The plate photo-bioreactor was used to cultivate *C. vulgaris*. Results showed that the culture of *C. vulgaris* could be used in a situation of catastrophic failure of higher plant under the emergencies. And the productivity could recover itself to the original state in 3 to 5 days to protect the system till the higher plant was renewed. Besides, *C. vulgaris* could grow well and the productivity could be affected by the light intensity which could help to keep the balance of CO_2 and O_2 in the IES efficiently. Thus, *C. vulgaris* could be included in the design of a BLSS as a "compensatory system" in the emergency contingency and a "regulator" during the normal maintenance.

Keywords: *Chlorella vulgaris*; Compensatory system; Regulator; Integrative experimental system; Plate photo-bioreactor; Multi-biological life support system experiments

1. Introduction

For long-term space exploration missions, it is necessary to establish the bioregenerative life support system (BLSS) which could produce food, revitalize water, and regenerate O_2 (Bartsev et al., 1996a; Lasseur et al., 1996; Sadeh and Sadeh, 1997). To achieve this goal, ground-based simulation experiments with human involved must be conducted firstly. Such work has been carried out in the Closed Ecology Experiment Facilities (CEEF), Biosphere-2, and BIOS (Allen and Nelson, 1999; Bartsev et al., 1996b; Morowitz et al., 2005; Nitta et al., 2000). Similar studies called "multi-biological life support system experiments" (MLSSE) have also been conducted in the present integrative experimental system (IES) (Hu et al., 2011; Tong et al., 2011).

In BLSS, higher plant, such as wheat and lettuce were cultivated for food supply and O_2 regeneration. However, there might be catastrophic failures of cultivating these higher plants under emergency conditions (Schneegurt et al., 1996). It could lead to a thorough imbalance of CO_2 and O_2, and may affect the security of BLSS making

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it take a long time for higher plants to be cultivated once again. Microalgae play an important role in gas controlling of the global ecosystem (Mike, 2009; Tsai et al., 2012). Unicellular *C. vulgaris* with typically rapid growth rate may quickly help to recover the system state after the emergency contingency. It may be functioned as a "compensatory system" to protect the system before the recovering of high plants.

Concentrations of CO_2 and O_2 could also be affected by various factors, such as the imbalances between the CO_2/O_2 gas exchange ratios of the heterotrophic and autotrophic components, which will inevitably lead to an unstable system and a loss of O_2 from the atmosphere (Schneegurt et al., 1996). In this case, one solution could be an adjustment of plant composition. But this approach requires a very long response time (30 to 60 days depending on plant types) to make a noticeable impact on the atmosphere composition (Gitelson and Okladnikov, 1996). In order to increase the stability of the system, *C. vulgaris* with an appropriate CO_2/O_2 gas exchange ratio could be introduced as a "regulator" to accommodate the gas balance.

Microalgae are candidates for inclusion in the BIOS and other ground-based experiments because of their typically rapid grow rates and easily controlled cultivation, as well as the compatible gas-exchange characteristics with human requirements (Ai et al., 2008; Ganzer and Messerschmid, 2009; Gitelson et al., 2003). But it caused digestive disturbances in human and animals when the microalgae constituted only a small fraction (1% or less) of the edible mass. It would be probably precluded from the life support food source (Gitelson et al., 2003). The biomass of microalgae seemed to be dead-end products in the closed system. Actually they could be either burned when the CO_2 concentration was low in the system, or fed to yellow mealworms (*Tenebrio molitor* L.) which were promising source of animal protein for human (Li et al., 2012). The yellow mealworms have good digestion and can even eat plastic. Thus the algae could participate in the food chain in the BLSS.

Although microalgae culture experiments have been widely conducted for O_2 regeneration, limited attention has been paid to the roles of "compensatory system" and "regulator" of BLSS.

The objective of this work was to demonstrate the ability of *C. vulgaris* culture to maintain stable CO_2 concentration with various conditions including light intensity reduction, temperature stress, CO_2 intermittent input, and simultaneous cultivation with silkworms.

2. Materials and methods

2.1. Algae and culture

C. vulgaris No. 7 was provided by the Freshwater Algae Culture Collection of Institute of Hydrobiology (FACHB, the Chinese Academy of Sciences) and cultivated with soil extract medium. The temperature was maintained at 25–30 °C. The culture was prepared for storage.

2.2. Culture medium

It proved that microalgae could grow in human urine to be partially involved into the human waste treatment in BLSS (Adamsson, 2000). Thus in this work synthetic human urine was used to cultivate *C. vulgaris* according to our previous study (Yang et al., 2008).

2.3. Experiment facilities

As shown in Fig. 1, the IES was composed of two parts: a CICS (Tong and Liu, 2011) and a plate photo-bioreactor. The gas leakage of the IES was about 0.0059% per day (Tong et al., 2011), which was negligible on the gaseous status of the system (Dempster et al., 2009). The plate photo-bioreactor was connected with the plant cultivating chamber and the animal breeding chamber of the CICS. In MLSSE, lettuce and silkworms were cultivated in the plant cultivating chamber and the animal breeding chamber, respectively, while *C. vulgaris* was grown in the plate photo-bioreactor. Four volunteers participated in this experiment for the gas exchange through a mask tube connected with the animal breeding chamber.

2.4. Photo-bioreactor

C. vulgaris was cultivated in the plate photo-bioreactor (Hu et al., 2012) in MLSSE. The effective volume was about 1.5 L with the size of 300 mm (H) × 400 mm (W) × 15 mm (D). Light source was composed of red ($\lambda = 660$ nm) and blue ($\lambda = 470$ nm) light emitting diodes (LEDs) with adjustable light intensities of 0–250 μmol m^{-2} s^{-1}. There was a light source on either side of the plate photo-bioreactor. Thus the plate photo bioreactor could be illuminated from both sides with an illumination area of 0.1 m^2 for each side. And the light intensity was calculated separately for each side of the plate photo-bioreactor.

2.5. Culture of C. vulgaris

C. vulgaris was previously cultivated in soil extract medium for seven days. Before inoculated into the plate photo-bioreactor, *C. vulgaris* solution was centrifuged and extracted by deionized water. This extraction repeated three times. Air from the plant cultivating chamber was pumped into the bottom of plate photo-bioreactor while the output air was returned to the animal breeding chamber.

A semi-continuous harvesting regime was employed. The supply of urine and the output of increment algae liquid were carried out by two peristaltic pumps. The influent rate of urine was confirmed by the biomass increment and N concentration in the plate photo-bioreactor.

During the first nine days, the samples taken for measuring the pH and biomass were returned to the reactor. On the tenth day, the system was stabilized; the medium taken for measure and evaporating water was compensated by deionized water.