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CO_2 Fixation by *Chlorella* KR-1 Using Flue Gas and its Utilization as a Feedstuff for Chicks

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Abstract A highly CO_2 tolerant microalga, *Chlorella* KR-1, has been isolated and used to fix CO_2 from actual flue gas. Growth of *Chlorella* KR-1 with the supply of flue gas from a liquified natural gas boiler was comparable to that obtained with 10% CO_2. *Chlorella* KR-1 produced from CO_2 fixation using the flue gas has about 50% crude protein with balanced amino acid profiles. Toxicity was not detected when the microalga was used as a feedstuff for chicks. These results indicate that the KR-1 cells could be a favorable protein source for poultry.

Key words: Biological CO_2 fixation, flue gas, *Chlorella* KR-1, feedstuff, poultry

Biological methods, particularly using microalgal photosynthesis, have several merits, such as mild conditions for CO_2 fixation and no requirements for the further disposal of trapped CO_2. Carbon fixed by microalgae is incorporated into carbohydrates and lipids for energy, and chemicals and foods can be produced from algal biomass.

If CO_2 fixation by microalgae is operated to recover CO_2 that has been emitted from industrial sources, the amount of algal cells produced from the CO_2 fixation process would be tremendous. Therefore, the strategy for utilizing algal cells is very important for an economically viable CO_2 fixation process [6]. Several studies have been carried out to utilize these algal cells that were produced from a CO_2 fixation process [5, 9, 12].

Recently, a few works for converting microalgae to a liquid fuel either by solvent extraction [3] or direct liquefaction [9] have been reported. However, the fuel production process from microalgae is only feasible for a certain algal species such as *Botryococcus braunii*, which has a high lipid content [9]. Green algae, including *Chlorella*, have been the subject of investigation as a possible source of food for animals and human beings, because they contain a high percentage of protein, minerals, and vitamins. Microalgae produced from wastewater treatment has been tested as a feedstuff for chicks and pigs [1,4,8]. Combs [2] reported that the substitution with 10% of *Chlorella* for soybean meal in the diet for chicks resulted in a remarkable improvement in growth and feed efficiency. Unfortunately, only a few works have been carried out with regards to the utilization of microalgae that was produced from CO_2 fixation as a feedstuff. Yanagi *et al.* [12] reported that *Chlorella* HA-1, cultured with 10% CO_2, contained a high nutritional value that was equivalent to the combined fodder made from crops, 80% corn flour and 20% soy bean. The work has been carried out with clean CO_2 rather than flue gas, and did not provide any experimental results in terms of the effect of algae on growth of animals.

Since actual flue gas contains various toxic compounds like SO_x, NO_x, and particulate matters, a direct introduction of the flue gas into the algal culture not only imposes extreme conditions on microalgae, but also the algae may contain toxic compounds in its body. Therefore, the toxicity test should be performed before using the algae as a feedstock. As of this time, no one had determined the effect of algal cells produced from CO_2 fixation, by using actual flue gas on the growth of animals.

The objective of this work was to develop a direct CO_2 recovery process by using *Chlorella* KR-1 from flue gas, and to determine the feasibility of KR-1 that was produced from the CO_2 fixation process as a feedstuff for chicks.

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MATERIALS AND METHODS

Strain and Culture Experiment

Chlorella sp. KR-1 that was isolated in Lee's laboratory, a highly CO_2 and NO_x tolerant, and fast growing strain, was used in this work [7, 10]. The strain was maintained on Detmer agar plate and cultured in a modified M4N medium. The media composition and the procedures were described elsewhere [11]. The initial pH of the medium was 5.3 and was not regulated.

Culture System and Growth Conditions

The growth tests of *Chlorella* KR-1 with two different gases (clean CO_2 properly diluted with air and actual flue gas from a boiler fueled by liquified natural gas (LNG)) were conducted in a small bioreactor setup. The flue gas contains about 10% CO_2 and 20–30 ppm NO_x, but no SO_x. All seed cultures were prepared with air-bubbling in the 10-l illuminated jar (8 l medium) at 25°C and 200 μmol/(m^2-sec). Air-grown cells were inoculated into the growth medium to obtain an initial cell concentration of 0.1 g/l. The bioreactors were illuminated by fluorescent tubes. The temperature of the cultures was maintained at 25°C and the light intensity in the surface of the reactor was 250 μmol/(m^2-sec). Samples were removed daily from the reactors to determine the algal growth.

All outdoor growth experiments were conducted to determine the cultural characteristics of *Chlorella* KR-1. Five 13-l bubble column reactors made of glass [11 cm (inner diameter), 150 cm in length] were used to culture *Chlorella* KR-1. The air sparger with an average pore size of 50 μm was located at the bottom of the reactor. Actual flue gas emitted from the boiler was directly sparged into the reactor. The reactors were then placed in a single straight array about 6 cm apart in a metal frame. The temperature and pH of the culture were not regulated. The gas flow rate was fixed at 0.2 vol gas/vol liquid/min. For the comparison work, flat plate photobioreactors (120 cm×80 cm×3 cm) were used for the outdoor culture of *Chlorella* KR-1.

Chick Studies

A total of 60 male Hy-line broiler chicks were housed and raised in cages with wire mesh floors. The chicks were fed after a practical diet for one week. Both control and 1% *Chlorella* KR-1 supplemented diet were fed for three weeks. During 4 weeks of growth assay, feed and water were supplied *ad libitum* and body weight and feed consumption were measured every week. The composition of the basal diet, expressed as a percentage, was as follows: yellow corn, 48.9; wheat, 23.0; wheat bran, 10.9; soybean meal, 7.1; rapeseed meal, 2.5; gluten feed, 2.0; fish meal, 1.5; tri-calciumphosphate, 1.1; animal fat; 0.6; vitamine mixture, 0.13; choline-Cl, 0.05; other trace elements to 100. At the end of the feeding trial, the foot color score was visually inspected by reference with Roche color standards (Hoffmann-La Roche Co. Ltd., Basle, Switzerland). Twenty scores per each treatment were summed up, and the average score was reported.

Assays

Algal growth was determined by measuring the absorbance level at 660 nm by using a spectrophotometer (UV-1601, Shimadzu Inc., Kyoto, Japan) and by converting into a dry cell weight. Light intensities were measured by using a quantum meter (LI-250, Li-Cor Inc., Lincoln, NE, U.S.A.), CO_2 concentrations were monitored by using a CO_2 analyzer (IR8400, Summit Analyzers Inc., Cleveland, OH, U.S.A.), and NO_x concentrations in a flue gas were measured by an NO_x analyzer (NONOXOR II, Bacharach Inc., Pittsburgh, PA, U.S.A.). Amino acid composition of KR-1 was analyzed by an amino acid analyzer (AA-404, Waters Inc., Milford, MA, U.S.A.), and fatty acid composition was determined by gas chromatography, equipped with a flame ionization detector (HP5890, Hewlett-Packard Inc., U.S.A.).

RESULTS AND DISCUSSION

Growth of *Chlorella* KR-1

Chlorella KR-1 was cultured with 10% CO_2 and the flue gas from a LNG fuelled boiler. As shown in Fig. 1, *Chlorella* KR-1 cultured with the supply of flue gas exhibited comparable growth with KR-1 cultured with 10% CO_2. Therefore, KR-1 could be applied for the direct recovery of CO_2 from stack gas that was emitted from the LNG boiler.

To investigate the CO_2 fixation rates by *Chlorella* KR-1 cultured outdoors, a series of experimental cultivations in both the tubular reactor and plate type photobioreactors

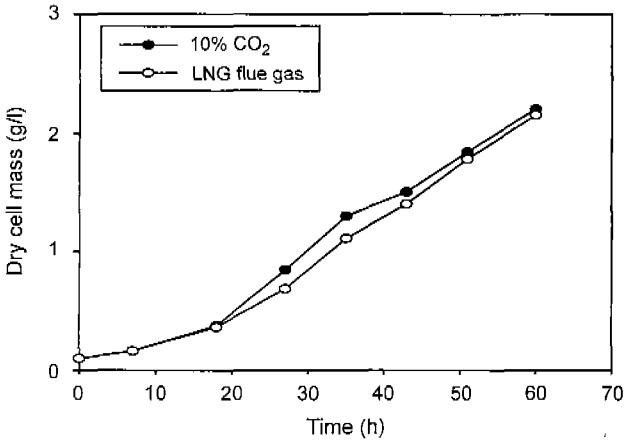

Fig. 1. Growth of *Chlorella* KR-1 cultured with CO_2 enriched air (10%) and LNG flue gas.
The cultures were illuminated at 250 μmol/(m^2-scc) and bubbled at 25°C. Gas flow rate was 0.5 vol gas/vol liquid/min.

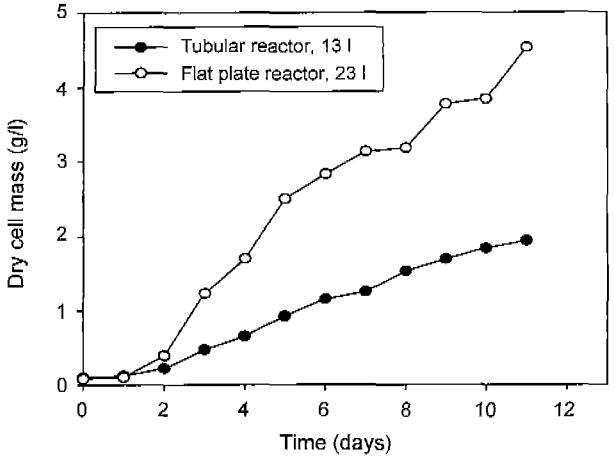

Fig. 2. Typical productivity curves of *Chlorella* KR-1 grown outdoors in two integrated culture systems (Tubular reactor and Flat plate reactor).
Gas flow rate was 0.2 vol gas/vol liquid/min.

Table 2. Amino acid composition of *Chlorella* KR-1 produced by using LNG flue gas.

Amino acid	Contents, mg/100 g
Asp	3,317.46
Glu	4,569.45
Ser	1,028.85
His	347.37
Gly	2,270.56
Thr	1,761.41
Ala	4,100.90
Arg	3,604.50
Tyr	1,085.61
Cys	179.13
Val	2,056.63
Met	341.56
Phe	1,921.00
Isol	1,310.00
Leu	3,375.37
Lys	1,632.69
Pro	3,766.89

have been carried out. Figure 2 shows a typical time course for the cell growth in the photobioreactors. In the tubular reactor, the cell concentration steadily increased and reached to 1.94 g/l after 11 days of cultivation, and this was 19 times higher than that of the inoculum cell mass. The linear growth rate of KR-1 under the cultural conditions was about 0.2 g/l/day. The growth rate was drastically reduced when the cell concentration was higher than 2 g/l. This shows that light limitation occurred in the tubular reactor. To enhance the light utilization efficiency, a plate type reactor with a shorter light path was employed. The final cell concentration and the linear growth rate of KR-1 cultured in the photobioreactor were 4.5 g/l and 0.6 g/L/day after 11 days of cultivation, respectively. The alga showed a stable linear growth rate even at the end of the culture.

Nutritional Value of *Chlorella* KR-1

The protein and lipid contents of *Chlorella* KR-1 cells cultured with either CO_2 enriched air (10%) or LNG flue gas were compared. As shown in Table 1, KR-1 cells contained 53–57% of protein and 4-6% of lipids. The protein content of KR-1 cells cultured with LNG flue gas was slightly higher than that of KR-1 supplied with CO_2 enriched air (10%). Unfortunately, the reason for the difference is still unclear. The nutrient contents of KR-1 cells were similar to those of *Chlorella* cells that were reported in the literature [12]. The *in vitro* digestibility (by pepsin) of KR-1 cells cultured with LNG flue gas was about 70%, which was much higher than that of KR-1 cultured with CO_2 enriched air.

The digestibility of KR-1 was rather low but similar to those of *Chlorella* sp. reported in the literature [12]. The low digestibility was mainly due to its thick cell wall. Some methods of pretreatment to enhance the digestibility have been studied in Ohh's laboratory, and the nutritional quality of a protein in *Chlorella* KR-1 was also evaluated by analyzing its amino acid profile. An amino acid profile of the dried *Chlorella* KR-1 cultured in LNG flue gas is given in Table 2, and it is quite similar to the amino acid values of the cells grown elsewhere [4]. The fatty acid content of KR-1 grown with LNG flue gas was also determined (Table 3). KR-1 had high levels of palmitic

Table 1. Comparison of chemical composition of *Chlorella* KR-1 produced from 10% CO_2 and LNG flue gas.

Components	10% CO_2	LNG flue gas
Crude Protein (Nx6.25)	52.9	57.4
Ether extracts	4.0	6.2
Carbohydrates	31.2	25.3
Ash	11.9	11.1
Pepsin digestibility	51.6	69.9
Calorific value, cal/g	4,850.0	5,091.0

Table 3. Fatty acid composition of *Chlorella* KR-1 produced by using LNG flue gas.

Fatty acid	Contents, %
$C_{14:0}$	4.98
$C_{16:0}$	29.72
$C_{16:1}$	2.54
$C_{18:0}$	7.00
$C_{18:1}$	15.18
$C_{18:2}$	21.51
$C_{18:3(\gamma)}$	1.30
$C_{18:3(\alpha)}$	7.11
$C_{20:1n9}$	2.53
$C_{20:4}$	3.12
$C_{20:5}$	1.10

Table 4. Quantitative evaluation of broilers fed on control and algal-containing diets.

Criteria	Diets	
	Control	Algal diet
Weight gain, g	1,259	1,246
FCE[1]	1.91	1.89
Foot color score[2]	2.3[3]	3.7[3]

1: Feed conversion efficiency (feed intake/weight gain). 2: Foot color score is an average value of 20 visual inspection scores by 4 panels, referred Roche standard color plate. Higher number indicates more yellowish color out of 1 (pale yellow) to 15 (reddish yellow) scale. 3. Values are from statistical analysis ($p<0.05$).

acid (16:0), and oleic (18:1) and linoleic acids (18:2), one of the essential fatty acids.

The body weight gain and feed conversion efficiency (FCE) of broilers fed either the control or algal diets for 3 weeks are summarized in Table 4. There was no difference ($p<0.05$) in body weight gain of birds fed either algal diet or control diet. However, the feed conversion efficiency (FCE) of the *Chlorella* supplemented diet was numerically improved compared to the control diet. In addition, there was yellowish color pigmentation on the foot of broilers raised on algal diet, which was attributable to a high content of carotenoids in the *Chlorella*. The deeper colour generally provides better consumer attraction compared to that from broilers of the control group.

Chlorella KR-1 could be applied to recover CO_2 directly from actual flue gas. The alga cells produced from the CO_2 fixation process contained relatively higher nutritional value that could be used as an efficient feedstuff for animals. However, the digestibility of KR-1 was too low to be used directly. Therefore, it is necessary to carry out further research to enhance the digestibility.

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References

1. Becker, E. W. 1994. *Microalgae,* pp. 196–249. Cambridge University Press, Cambridge, U.K.
2. Combs, G. F. 1952. Alga (*Chlorella*) as a source of nutrients for the chick. *Science* **116:** 453–454.
3. Frenz, J., C. Largeau, E. Casadevall, F. Kollerup, and A. J. Daugulis. 1989. Hydrocarbon recovery and biocompatibility of solvents for extraction from cultures of *Botrycoccus braunii. Biotechnol. Bioeng.* **34:** 755–762.
4. Grinstead, G. S., M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. L. Nelssen. 2000. Effects of *Spirulina platensis* on growth of weanling pigs. *Animal Feed Sci. Technol.* **83:** 237–247.
5. Honnami, K., A. Hirano, S. Kunito, T. Tsuyuki, T. Kinoshita, and Y. Ogushi. 1997. A new marine microalga cultivation in a tubular bioreactor and its utilization as an additive for paper surface improvements. *Energy Convers. Mgmt.* **38:** 481–486.
6. Benemann, J. R. 1997. CO_2 mitigation with microalgae system. *Energy Convers. Mgmt.* **38:** 475–479.
7. Lee, J. H., J. S. Lee, C. S. Shin, C. S. Park, and S. W. Kim. 2000. Effect of NO and SO_2 on growth of highly CO_2 tolerant microalgae. *J. Microbiol. Biotechnol.* **10:** 338–343.
8. Leveille, G. A., H. E. Sauberlich, and J. W. Shockley. 1962. Protein value and the amino acid deficiencies of various algae for growth of rats and chicks. *J. Nutrition* **76:** 423–428.
9. Sawayama, S., T. Minowa, and S. Yokoyama. 1999. Possibility of renewable energy production and CO_2 mitigation by thermochemical liquefaction of microalgae. *Biomass and Bioenergy* **17:** 33–39.
10. Sung, K. D., J. S. Lee, C. S. Shin, and S. C. Park. 1999. Isolation of a new highly CO_2 tolerant fresh water microalga, *Chlorella* sp. KR-1. *Renewable Energy* **16:** 1019–1022.
11. Sung, K. D., J. S. Lee, C. S. Shin, and S. C. Park. 1998. Enhanced cell growth of *Chlorella* sp. KR-1 by the addition of iron and EDTA. *J. Microbiol. Biotechnol.* **8:** 409–411.
12. Yanagi, M., Y. Watanabe, and H. Saiki. 1995. CO_2 fixation by *Chlorella* sp. HA-1 and its utilization. *Energy Convers. Mgmt.* **36:** 713–716.