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High Photosynthetic Productivity of Green Microalga *Chlorella sorokiniana*

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Abstract

The batch culture of a newly isolated strain of a green microalga, *Chlorella sorokiniana*, was carried out using a conical helical tubular photobioreactor. The isolate was capable of good growth at 40°C under an airstream enriched with 10% CO_2. The maximum photosynthetic productivity was 34.4 g of dry biomass/(m^2 of installation area · d) (12-h light/12-h dark cycle) when the cells were illuminated with an average photosynthetic photon flux density (photosynthetically active radiation ([PAR] 400–700 nm) simulating the outdoors in central Japan (0.980 mmol photons/[m^2·s]). This corresponded to a photosynthetic efficiency of 8.67% (PAR), which was defined as the percentage of the light energy recovered as biomass (394 kJ/[reactor·d]) to the total light energy received (4545 kJ/[reactor·d]). A similarly high photosynthetic efficiency (8.12% [PAR]) was also attained in the combined presence of 10% CO_2, 100 ppm of NO, and 25 ppm of SO_2. Moreover, good photosynthetic productivity was also obtained under high temperature and high light intensity conditions (maximum temperature, 46.5°C; 1.737 mmol photons/[m^2·s]), when simulating the strong irradiance of the midday summer sun. This strain thus appears well suited for practical application for converting CO_2 present in the stack gases emitted by thermal power plants and should be feasible even during the hot summer weather.

Index Entries: Microalgae; carbon dioxide; global warming; photosynthesis; bioreactor; effective utilization.

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Introduction

Microalgae have a greater capacity for photosynthesis than higher plants and are capable of synthesizing a variety of valuable substances *(1)*. This characteristic makes them potentially useful as part of a CO_2 fixation system for converting CO_2 present in industrial waste gases, such as stack gases from thermal power plants, into valuable microalgal biomass. In that regard, we have engineered a conical helical tubular photobioreactor (HTP) *(2)* capable of the high photosynthetic productivity required for such a process to be practical. The unique shape of this photobioreactor enables it to effectively gather photosynthetically active solar radiation and convert the light energy to chemical energy in the form of biomass. Because of its relatively high efficiency, this photobioreactor is especially productive during the spring and autumn, when solar radiation and ambient temperatures are below their peaks. In summer, photosynthetic productivity is diminished owing to excessive heating of the culture medium. Unfortunately, because a cooling system adds substantially to the production cost, the high temperatures in summer are one of the main factors restricting photosynthetic productivity in closed-type photobioreactors *(3)*. It would thus be advantageous to obtain a strain of microalgae that is tolerant to high temperatures and can provide efficient photosynthesis during summer without the use of a cooling system.

Because *Chlorella* is extensively used commercially, we were interested in it as the microalgae for biomass production from CO_2. A high-temperature species, *Chlorella sorokiniana*, was isolated by Sorokin and Myers *(4)* in 1953; it grows at temperatures up to 38–42°C *(5)*, and this is the only known species of *Chlorella* that tolerates such temperatures *(6)*. An additional prerequisite of the *Chlorella* species, with respect to the treatment of the stack gases from thermal power plants, is its resistance to high concentrations of CO_2, NO_X, and SO_X. Microalgae capable of growing under high concentrations of CO_2 have been successfully screened and isolated using CO_2-enriched air *(7–9)*. A similar investigation was also used to screen NO-tolerant strains *(10)*, and, indeed, NO removal by microalgae from stack gases was recently tested using a tubular photobioreactor *(11)*. On the other hand, the effect of SO_2 on the growth of the microalgal strains tested has been a strong inhibition owing to acidification of the culture medium *(10)*.

In the present study, we have newly isolated *C. sorokiniana* Shihira et Krauss *(12)*, and examined its growth characteristics when exposed to high concentrations of CO_2, NO_X, and SO_X, and evaluated its photosynthetic capacity in our conical HTP.

Materials and Methods

Microalga

The microalga used in this study was isolated from a hot spring at Oowakudani, Kanagawa Prefecture in Japan. Isolation was carried out from

an enrichment culture under a 10% CO_2-enriched air-flowing condition at 40°C. An illumination of 0.030 mmol photons/(m^2·s) was provided all day using white fluorescent lights for the isolation. To ensure its purity, bacterial contamination of the isolate was checked using the dilution agar plate method and microscopic observations. This isolate was named the HO-1 strain.

Micrograph Observation and DNA Sequence

The cultured cells were harvested by centrifugation, fixed with 2% glutaraldehyde in 0.05 *M* phosphate buffer for 2 h at 4°C, and postfixed with 2% osmium tetraoxide in 0.05 *M* phosphate buffer for 2 h at 4°C. The fixed material was then dehydrated using a graded acetone series and embedded in Spurr's resin. The preparation was then stained with 4% uranyl acetate, followed by 0.4% lead citrate, and examined under a transmission electron microscope (H-7500; Hitachi, Tokyo, Japan).

Total DNA was isolated from the tested strain using an ISOPLANT kit (Nippon Gene, Toyama, Japan). The 18S rRNA coding region was amplified from the bulk genomic DNA by polymerase chain reaction using Ex Taq DNA polymerase (Takara Shuzo, Kyoto, Japan) and synthetic oligonucleotide primers (F5'-AACCTGGTTGATCCTGCCAGTAGTC-3'; R5'-TTGATCCTTCTGCAGGTTCACCTAC-3'). The amplified product was then ligated into the pGEM-T expression vector (Promega, Madison, WI) using *Escherichia coli* JM109 as a host for the vector. After cloning, the plasmid DNA containing the 18S rRNA coding region was prepared using a Plasmid Miniprep kit (Bio-Rad, Hercules, CA), and its complete sequence was determined using a DNA sequencing system (373S; Perkin-Elmer, Foster City, CA).

Culture Experiment

The growth characteristics of the tested strain were examined using flat culture bottles in triplicate. Three-day batch cultures were carried out using various CO_2 concentrations, temperatures, and initial pH values. The effects of NO and SO_2 were also investigated under an atmosphere of 10% CO_2-enriched air at 35°C. Illumination at 0.120 mmol photons/(m^2·s) on the surface of flat culture bottles was provided 12 h/d using white fluorescent lights under all conditions tested. The cell density of the inoculum was 0.05 g of dry biomass/L. A total of 100 mL of MBM medium *(9)* was used, and the airflow rate was 40 mL/min. The medium was sampled every 12 h for the analyses.

Figure 1 is a schematic depiction of the conical HTP system. The unit size and lamps used as the illumination system were different from the conical HTP system described in a previous report *(2)*. We used the upside-down conical HTP system in this experiment because it could gather and use reflected light energy *(2)*. On the other hand, the upright one was deemed to reflect irradiation outside. The conical HTP used to investigate

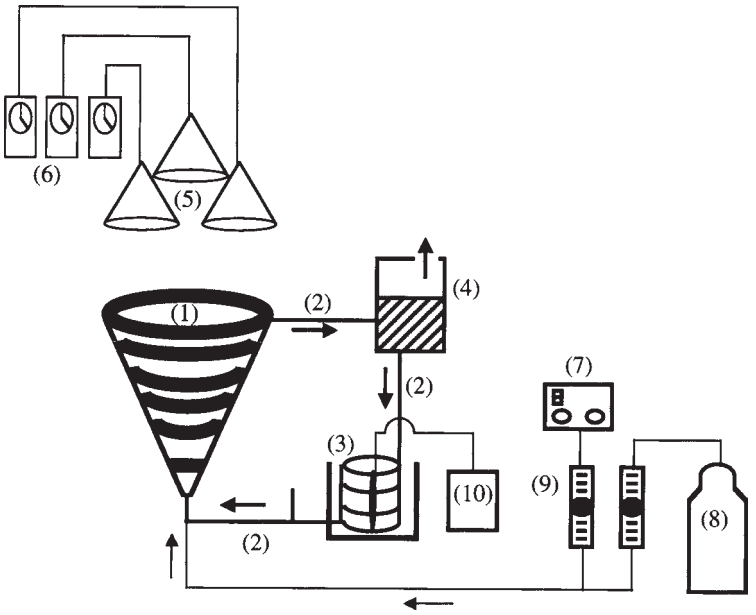

Fig. 1. Schematic depiction of the conical HTP system, which is comprised of ten parts: 1, conical photostage made of transparent polyvinyl chloride (PVC) tubing (internal diameter, 16 mm; wall thickness, 2 mm; cone angle, 60°); 2, passage (PVC tubing) for circulating liquid medium; 3, heat exchanger consisting of 11.6 m of PVC tubing set in a water bath; 4, degasser; 5, three metal halide lamps; 6, three timers; 7, air pump; 8, CO_2 gas cylinder; 9, gas flowmeter; and 10, cooling device.

the photosynthetic productivity in our study had an installation area of 0.5 m^2. The interior of the photostage was illuminated at a 60° angle over its entire 1.0-m^2 area using either 250 or 400 W metal halide lamps depending on the desired illumination conditions. The average light intensities flush with the top photostage and at the light receiving area were determined from respective measurements of 120 and 248 selected points made with a quantum meter (LI-189; LI-COR). When illuminated with the 250-W lamps, the average light intensity flush with the top photostage was 0.980 mmol photons/(m^2·s), which corresponds to the average photosynthetic photon flux density outdoors between April and September in central Japan. An average light intensity of 1.737 mmol photons/(m^2·s) was obtained with the 400-W lamps, which corresponds to the strong irradiance of the midday sun in the summer.

Batch cultures were carried out in the conical HTP for 6 d using 10% CO_2-enriched air supplied at various flow rates. The effects of NO and SO_2 on the photosynthetic productivity were investigated, as was the effect of the high light intensity (400-W lamps). The cell density of the inoculum added to the conical HTP, which was derived from cell samples originally cultured in the conical HTP, was 0.4 g of dry biomass/L. A total of 14 L of M4N medium *(9)* was used. The cultures were illuminated for 12 h/d, and the medium was sampled every 12 h for the analyses.

Analytical Methods

The dry weights of the tested strain samples in suspension were measured as a function of the absorbance at 750 nm using a spectrophotometer (DU650; Beckman, Urbana, IL). The relationship between dry weight and absorbance was as follows: Dry Weight (g/L) = $0.362 \times OD_{750}$ ($OD_{750} < 0.3$).

To determine carbon content, batch cultures were centrifuged, and the harvested cells were then washed, dried at 105°C for 24 h, and crushed into a powder. The carbon content in the powdered biomass was determined using an elemental analyzer (Sumigraph NC-800; Sumika, Osaka, Japan). The pH of the culture medium was measured with a pH sensor.

Results

Taxonomic Characteristics

Figures 2 and 3 show transmission electron micrographs of the isolated microalga. The microalga is unicellular and solitary. The cells are globular and 3–8 μm in diameter. The cell wall is composed of a one-layered envelope and is smooth on the surface. Each cell has a cup-shaped chloroplast and a pyrenoid. The pyrenoid is spherical to subspherical, divided into two parts by a single thylakoid and surrounded with divided starch sheaths. Vacuoles and lipid-like granules are present within the cells. The cells are capable of producing four to eight autospores, which are released by the breaking of the cell wall into several fragments.

The tested strain achieved an overall maximum specific growth rate in 5% CO_2 at 35°C and pH 6.0 (Fig. 4). The specific growth rate was defined as $\mu = (1/X) \times (dX/dt)$, in which X is the microalgal mass concentration (grams/liter), t is time (hours), and μ is specific growth rate ($hours^{-1}$). By the integration of this equation, the specific growth rate was calculated from time-dependent change of microalgal mass concentration in the batch culture. The maximum specific growth rate was the highest value of the individual daily specific growth rate. Although growth was slightly lower in the presence of 10 or 15% CO_2, the tested strain maintained a high growth potential over a wide range of CO_2 concentrations. It also exhibited a high maximum specific growth rate at 40°C; there was no growth at 50°C. Finally, the maximum growth potential was unaffected by pH values above 4.0, although it was drastically inhibited at pH 3.0.

Figure 5 shows the complete DNA sequence of the 18S rRNA coding region from the tested strain. The sequence was 1797 nucleotides long, its G+C content was 49.75 mol%, and its identity with the known sequence from another strain of *C. sorokiniana* (accession no. X74001 of EMBL Data Library) was 99.8%.

Growth Characteristics

Table 1 summarizes the effects of NO and SO_2 on the growth of the tested strain. The maximum specific growth rates and final dry weights

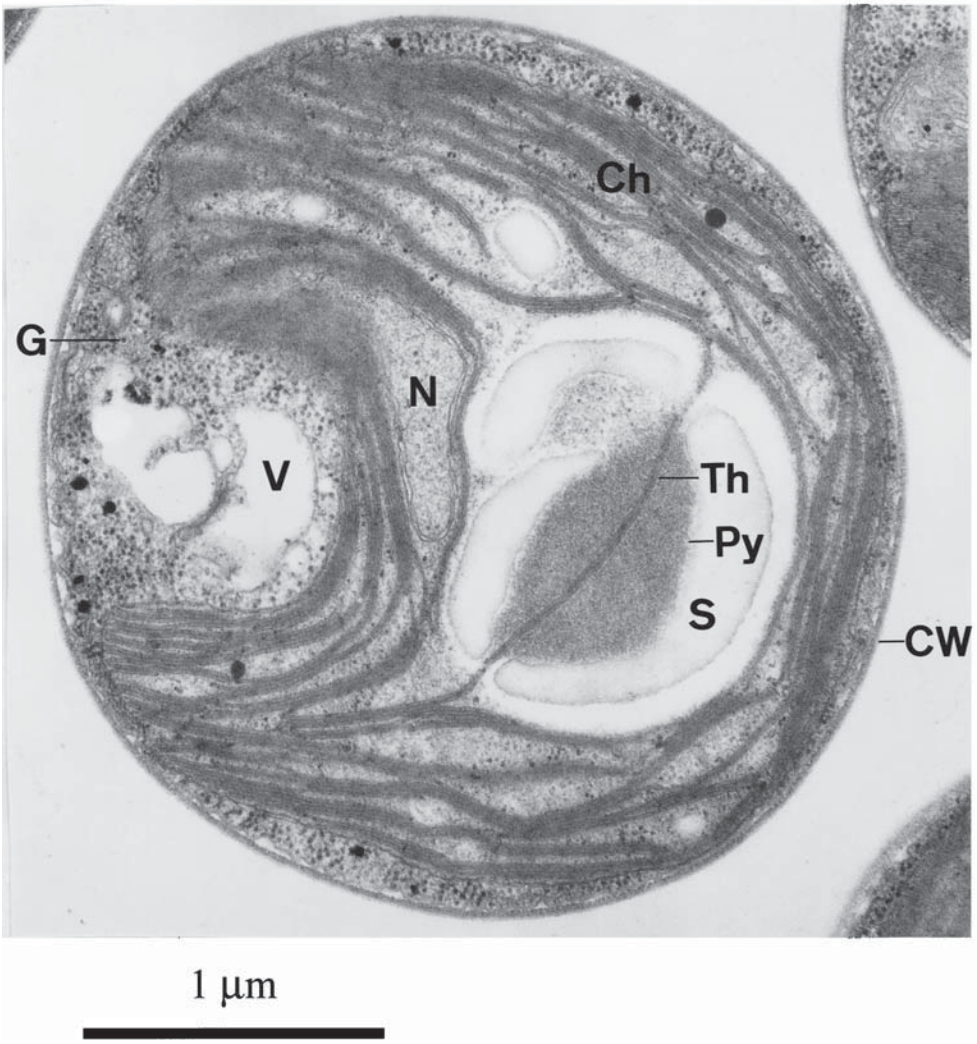

Fig. 2. Transmission electron micrograph of *C. sorokiniana* strain HO-1 showing cellular structure; Ch, chloroplast; CW, cell wall; G, granule; N, nucleus; Py, pyrenoid; S, starch; Th, thylakoid; V, vacuole.

were almost equal under all conditions tested. In the presence of a 10% CO_2-enriched airstream containing 100 ppm of NO and 25 ppm of SO_2, the maximum specific growth rate was 0.111 h^{-1}. On the other hand, the tested strain could not survive in the presence of 40–60 ppm of SO_2 owing to acidification of the culture medium. The cells turned white and died as the pH declined to approx 3.6; a similar effect was seen in batch cultures when the initial pH was 3.0.

Figure 6 depicts the time-dependent changes in the pH of the culture medium in the presence of selected concentrations of SO_2, with or without the tested strain. In the absence of the tested strain, SO_2 caused a dramatic,

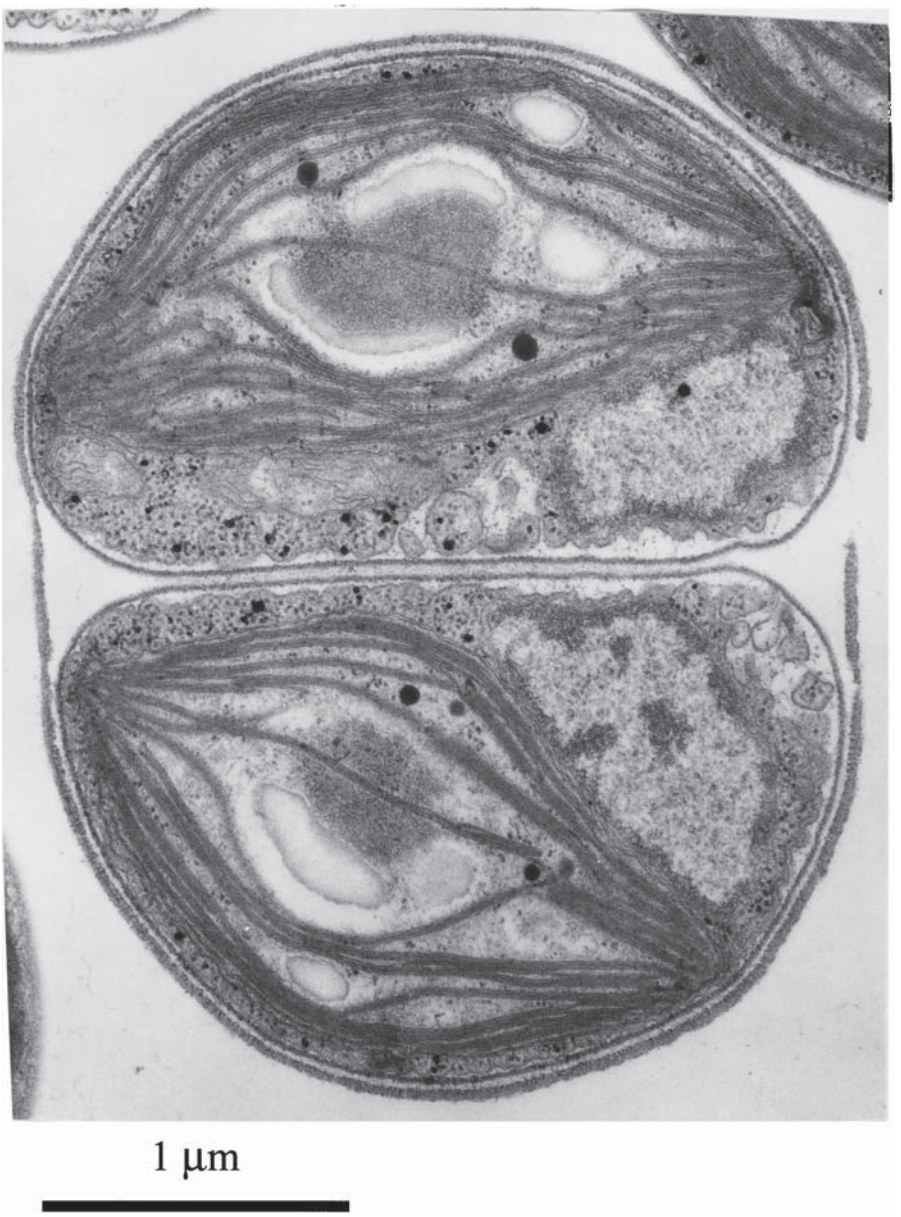

Fig. 3. Transmission electron micrograph of *C. sorokiniana* strain HO-1 showing the formation of aplanospore.

concentration-dependent decline in pH from 6.0 to 3.0. As already mentioned, airstreams containing 40–60 ppm of SO_2 similarly acidified the culture medium even when the cells were present. However, when the tested strain was present and the SO_2 concentrations were 25 ppm or less, the pH declined from 6.0 to 5.0 within 30 min but then gradually returned to 6.0.

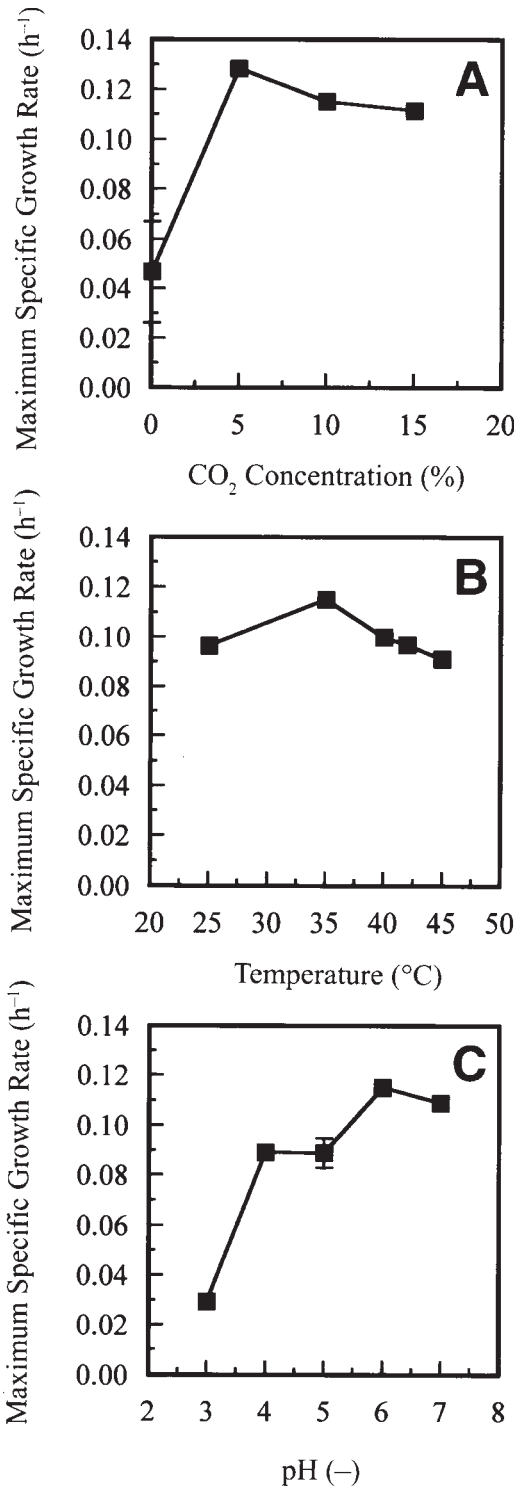

Fig. 4. Effect of **(A)** CO_2 concentration, **(B)** temperature, and **(C)** initial pH on maximum specific growth rate of *C. sorokiniana* strain HO-1. Vertical lines indicate SEM.

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aacctggttg atcctgccag tagtcATATG CTTGTCTCAA AGATTAAGCC ATGCATGTCT    60
AAGTATAAAC TGCTTTATAC TGTGAAACTG CGAATGGCTC ATTAAATCAG TTATAGTTTA   120
TTTGATGGTA CCTACTACTC GGATACCCGT AGTAAATCTA GAGCTAATAC GTGCGTAAAT   180
CCCGACTTCT GGAAGGGACG TATTTATTAG ATAAAAGGCC GACCGGGCTC TGCCCGACTC   240
GCGGTGAATC ATGATAACTT CACGAATCGC ATGGCCTTGT GCCGGCGATG TTTCATTCAA   300
ATTTCTGCCC TATCAACTTT CGATGGTAGG ATAGAGGCCT ACCATGGTGG TAACGGGTGA   360
CGGAGGATTA GGGTTCGATT CCGGAGAGGG AGCCTGAGAA ACGGCTACCA CATCCAAGGA   420
AGGCAGCAGG CGCGCAAATT ACCCAATCCT GACACAGGGA GGTAGTGACA ATAAATAACA   480
ATACTGGGCC TTTTCAGGTC TGGTAATTGG AATGAGTACA ATCTAAACCC CTTAACGAGG   540
ATCAATTGGA GGGCAAGTCT GGTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAT   600
ATTTAAGTTG CTGCAGTTAA AAAGCTCGTA GTTGGATTTC GGGTGGGGCC TGCCGGTCCG   660
CCGTTTCGGT GTGCACTGGC AGGGCCCACC TTGTTGCCGG GGACGGGCTC CTGGGCTTCA   720
CTGTCCGGGA CTCGGAGTCG GCGCTGTTAC TTTGAGTAAA TTAGAGTGTT CAAAGCAGGC   780
CTACGCTCTG AATACATTAG CATGGAATAA CACGATAGGA CTCTGGCCTA TCCTGTTGGT   840
CTGTAGGACC GGAGTAATGA TTAAGAGGGA CAGTCGGGGG CATTCGTATT TCATTGTCAG   900
AGGTGAAATT CTTGGATTTA TGAAAGACGA ACTACTGCGA AAGCATTTGC CAAGGATGTT   960
TTCATTAATC AAGAACGAAA GTTGGGGGCT CGAAGACGAT TAGATACCGT CCTAGTCTCA  1020
ACCATAAACG ATGCCGACTA GGGATCGGCG GATGTTTCTT CGATGACTCC GCCGGCACCT  1080
TATGAGAAAT CAAAGTTTTT GGGTTCCGGG GGGAGTATGG TCGCAAGGCT GAAACTTAAA  1140
GGAATTGACG GAAGGGCACC ACCAGGCGTG GAGCCTGCGG CTTAATTTGA CTCAACACGG  1200
GAAAACTTAC CAGGTCCAGA CATAGTGAGG ATTGACAGAT TGAGAGCTCT TTCTTGATTC  1260
TATGGGTGGT GGTGCATGGC CGTTCTTAGT TGGTGGGTTG CCTTGTCAGG TTGATTCCGG  1320
TAACGAACGA GACCTCAGCC TGCTAAATAG TCACGGTTGG TTCTCCAGCC GGCGGACTTC  1380
TTAGAGGGAC TATTGGCGAC TAGCCAATGG AAGCATGAGG CAATAACAGG TCTGTGATGC  1440
CCTTAGATGT TCTGGGCCGC ACGCGCGCTA CACTGATGCA TTCAACGAGC CTAGCCTTGG  1500
CCGAGAGGCC CGGGTAATCT TTGAAACTGC ATCGTGATGG GGATAGATTA TTGCAATTAT  1560
TAATCTTCAA CGAGGAATGC CTAGTAAGCG CAAGTCATCA GCTTGCGTTG ATTACGTCCC  1620
TGCCCTTTGT ACACACCGCC CGTCGCTCCT ACCGATTGGG TGTGCTGGTG AAGTGTTCGG  1680
ATTGGCGACC GGGTGCGGTC TCCGCTCTCG GCCGCCGAGA AGTTCATTAA ACCCTCCCAC  1740
CTAGAGGAAG GAGAAGTCGT AACAAGGTTT CCgtaggtga acctgcagaa ggatcaa     1797
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Fig. 5. Complete DNA sequence of the 18S rRNA coding region from *C. sorokiniana* strain HO-1.

Table 1
Effects of NO and SO_2 on Growth of *C. sorokiniana* Strain HO-1

Flowing gas composition	Maximum specific growth rate (h^{-1}) (mean ± SE)	Final dry wt (g/L) (mean ± SE)
A. 10% CO_2 in air	0.115 ± 0.001	1.68 ± 0.02
B. 10% CO_2 + 100 ppm NO in air	0.110 ± 0.000	1.53 ± 0.03
C. 10% CO_2 + 15 ppm SO_2 in air	0.115 ± 0.002	1.57 ± 0.02
D. 10% CO_2 + 25 ppm SO_2 in air	0.109 ± 0.004	1.71 ± 0.04
E. 10% CO_2 + 100 ppm NO + 25 ppm SO_2 in air	0.111 ± 0.001	1.53 ± 0.04

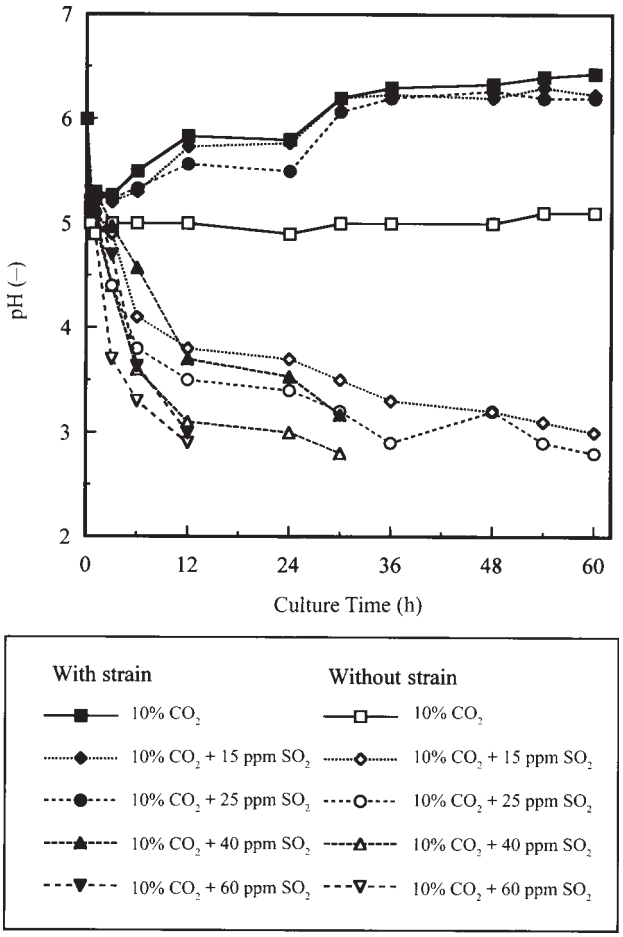
7
6
5
4
3
2
pH (–)
0
12
24
36
48
60
Culture Time (h)
With strain
Without strain
10% CO2
10% CO2 + 15 ppm SO2
10% CO2 + 25 ppm SO2
10% CO2 + 40 ppm SO2
10% CO2 + 60 ppm SO2
10% CO2
10% CO2 + 15 ppm SO2
10% CO2 + 25 ppm SO2
10% CO2 + 40 ppm SO2
10% CO2 + 60 ppm SO2

Fig. 6

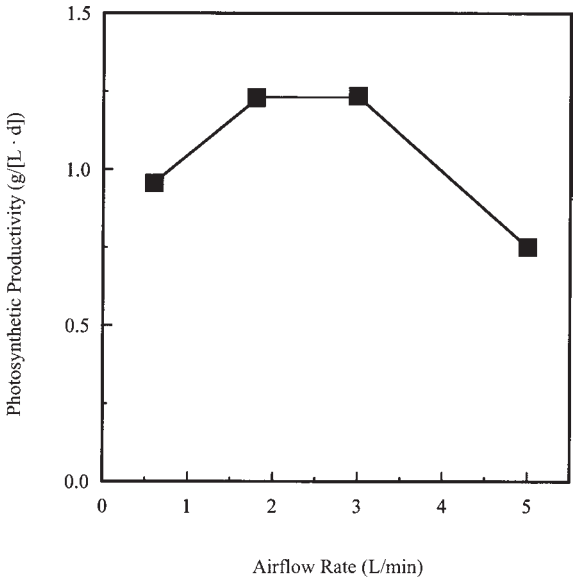
1.5
1.0
0.5
0.0
Photosynthetic Productivity (g/[L · d])
0
1
2
3
4
5
Airflow Rate (L/min)

Fig. 7

Photosynthetic Performance in the Conical HTP

Figure 7 shows the photosynthetic productivities of the tested strain under different airflow rates in the conical HTP. The HTP maintained high photosynthetic productivity over a wide range of airflow rates (0.6–5.0 L/min). Because the 10% CO_2-enriched air flowing at 1.8 L/min proved to be optimal for the photosynthetic productivity, subsequent experiments were conducted using this value.

Table 2 summarizes the effects of NO and SO_2 on the growth of the tested strain in the conical HTP. The photosynthetically active radiation ([PAR]; 400–700 nm) input to the photobioreactor was calculated to be 0.484 mmol photons/(m^2·s) (mean of the light receiving area) × 1.0 m^2 (illuminated inside surface of the photostage) ÷ 0.0046 = 105.2 (J/s or W), in which 0.0046 is the conversion factor from W/m^2 to mmol photons/(m^2·s) in the metal halide lamps *(13)*. The total energy input over a day was calculated to be 105.2 (J/s) × 60 × 60 × 12 = 4545 kJ/(reactor·d) (12-h light/12-h dark cycle). The maximum photosynthetic productivity was the highest value of the individual daily photosynthetic productivity in the batch culture. The maximum photosynthetic productivity per installation area was 34.4 g of dry biomass/(m^2 of installation area · d) in the 10% CO_2-enriched air under an average photosynthetic photon flux density simulating the outdoors in central Japan. Similar high photosynthetic productivity was obtained under the 10% CO_2-enriched air containing 100 ppm of NO and 25 ppm of SO_2. No cooling apparatus was used, and even though the temperature of the medium reached 42°C in every case, the tested strain showed a high growth potential.

The photosynthetic efficiency was defined as the percentage of the light energy recovered as biomass to the total light energy received. Photosynthetic efficiency was investigated to evaluate the utilization efficiency of the received light energy (Table 2). The carbon content in the powdered biomass was determined to be 48.1%, and since the chemical energy of the carbon was 47.7 kJ/g of carbon *(14)*, the energy of the total biomass was estimated to be 22.9 kJ/g of dry biomass. Thus, the maximum photosynthetic efficiency in the 10% CO_2-enriched air containing 100 ppm of NO and 25 ppm of SO_2 was calculated to be 32.2 g/(m^2·d) (maximum photosynthetic productivity per installation area) × 0.5 m^2/reactor (installation area) × 22.9 kJ/g of dry biomass (energy of biomass) = 369 kJ/(reactor·d) (maximum recovered light energy). Then, 369 kJ/(reactor·d) (maximum recovered light energy) ÷ 4545 kJ/(reactor·d) (received light energy) × 100 (percentage) = 8.12% (maximum photosynthetic efficiency). The maximum

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Fig. 6. *(previous page)* Time-dependent changes in the pH of the culture medium, elicited in the presence or absence of *C. sorokiniana* strain HO-1 by the indicated concentrations of SO_2 in 10% CO_2-enriched air. Vertical lines indicate SEM.

Fig. 7. *(previous page)* Effect of airflow rate on the photosynthetic productivity of *C. sorokiniana* strain HO-1 in a conical HTP under an average photosynthetic photon flux density simulating the outdoors of central Japan.

Table 2
Maximum Photosynthetic Productivity and Efficiency of *C. sorokiniana* Strain HO-1 Grown in Conical HTP Under Average Photosynthetic Photon Flux Density Simulating Outdoors of Central Japan

Flowing gas composition	Received light energy (kJ/[reactor·d]) (12-h light condition)	Maximum photosynthetic productivity (g/[L·d]) (12-h light condition)	Maximum photosynthetic productivity per installation area (g/[m^2·d]) (12-h light condition)	Maximum recovered light energy[a] (kJ/[reactor·d]) (12-h light condition)	Maximum photosynthetic efficiency (%) (PAR)
A. 10% CO_2	4545	1.23	34.4	394	8.67
B. 10% CO_2 + 100 ppm NO	4545	1.21	33.9	388	8.54
C. 10% CO_2 + 100 ppm NO + 25 ppm SO_2	4545	1.15	32.2	369	8.12

[a]Calculated from experimental data of carbon contents and calorific value for 1 g of carbon of microalgal biomass *(14)*.

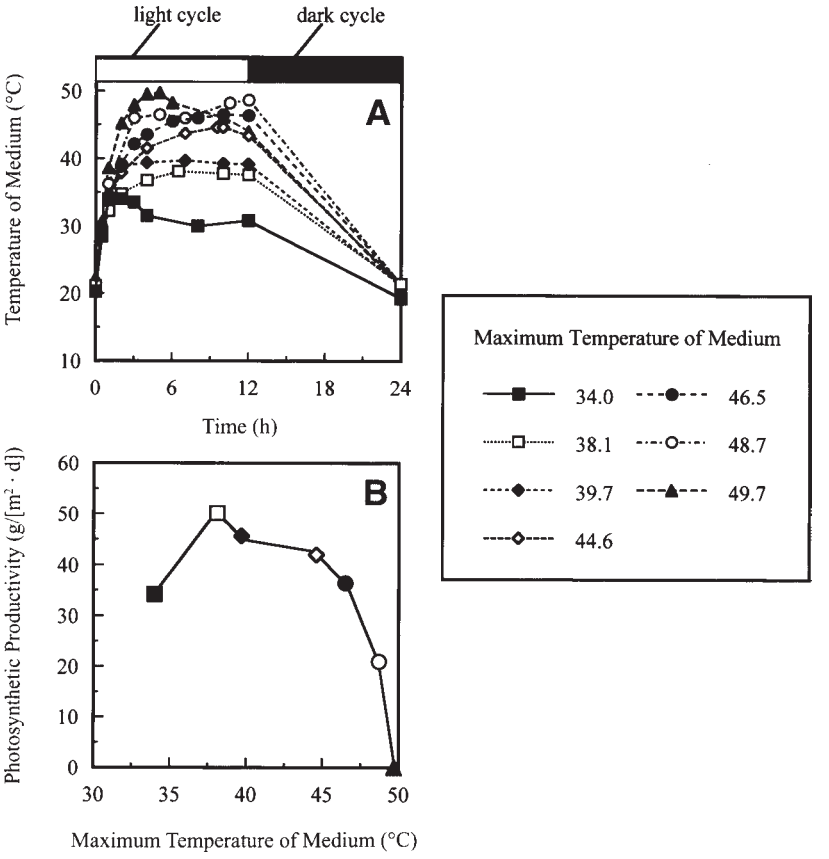

Fig. 8. **(A)** Time-dependent hourly changes in the temperature of the culture medium during 12-h light/12-h dark cycle; **(B)** effect of culture medium temperature on photosynthetic productivity of *C. sorokiniana* strain HO-1 grown in a conical HTP under a simulated midday, summer sun.

photosynthetic efficiency in 10% CO_2-enriched air was 8.67% (PAR), and the photosynthetic efficiency was maintained over 8% (PAR) in the presence of CO_2, NO, and SO_2.

To simulate the high light intensity of summer, batch cultures were grown under 400-W metal halide lamps in the conical HTP. For purposes of this experiment, a cooling apparatus was used to regulate the maximum temperature. Figure 8A shows the time-dependent hourly changes in the temperature of the culture medium during the 12-h light/12-h dark cycle. At the onset of illumination in each case, the temperature of the medium increased to nearly the designated maximum within 6 h and was maintained there for more than 6 h. Figure 8B shows the effect of the culture medium temperature on the photosynthetic productivity of the tested strain. Maximum photosynthetic productivity was achieved at 38.1°C, and the tested strain exhibited a good growth at temperatures as high as 46.5°C. The cells did not survive at 49.7°C, but lived and even grew at 48.7°C.

The average intensity of the light falling on the receiving area was 0.889 mmol photons/(m^2·s), and the received light energy was calculated to be 8351 kJ/(reactor·d). The maximum photosynthetic productivity per installation area was 50.1 g of dry biomass/(m^2 of installation area · d) in 10% CO_2-enriched air under strong illumination simulating that of the midday sun in the summer. The light energy recovered as biomass was calculated to be 574 kJ/(reactor·d), and the maximum photosynthetic efficiency achieved at the maximum temperature of 38.1°C was 6.87% (PAR).

Discussion

Taxonomic Identification

On the basis of the cellular details, the growth characteristics of the isolate at high temperature, and by comparison of the 18S rRNA gene with the known *Chlorella* species, the microalga used in this study was identified to be *C. sorokiniana* Shihira et Krauss *(12)*.

Growth Characteristics

The tested strain maintained a high growth potential under an airstream enriched with 10–15% CO_2, concentrations typical of the CO_2 content in stack gases from thermal power plants. No lag time before the onset of growth was noted at 40°C, which represents an advantage over another *Chlorella* strain with which there was a 5-d lag at 40°C *(7)*. Indeed, the strain appears well suited for growth in hot weather, and when grown outdoors in the conical HTP, a cooling apparatus is apparently unnecessary.

Negoro et al. *(10)* reported that a green alga, *Nannochloropsis* sp. NANNO$_2$, grew in the presence of NO after a lag period, but could not grow in the presence of SO_2. Compared with this strain, the growth of the tested strain was unaffected by 100 ppm of NO or 25 ppm of SO_2, which are actually the same levels as the NO and SO_2 concentrations in the stack gases of most modern thermal power plants in Japan, though the liquefied natural gas (LNG)-fired plants do not contain SO_2. After an early decrease, the tested strain maintained the pH of the culture medium at about 6.0 in the presence of 25 ppm of SO_2 or less. More important, the *C. sorokiniana* strain HO-1 was also able to maintain a pH near 6.0 in the presence of a mixture containing CO_2, NO, and SO_2, which means that it is potentially applicable to oil- or coal-fired power plants as well as LNG-fired plants.

Photosynthetic Productivity in the Conical HTP

We assessed the photosynthetic performance of the conical HTP in terms of the absolute quantity of biomass produced. Using *Spirulina platensis* during the summer in Italy, the photosynthetic productivities of 18 and 24 g/(m^2·d) were reported with vertical panel and sun-oriented panel

reactors, respectively *(3)*. Vonshak and Guy *(15)* reported that in Israel the photosynthetic productivity of *S. platensis* was 20.8 g/(m^2·d) in an outdoor pond equipped with a paddle wheel. The photosynthetic productivity of the tested strain grown in our conical HTP was 34.4 g of dry biomass/(m^2 of installation area · d) under an average photosynthetic photon flux density simulating the outdoors in central Japan, which is higher than has been obtained with other culture systems *(3,15–17)*. This value was higher than the photosynthetic productivity of 21.5 g of dry biomass/(m^2 of installation area · d) using the *Chlorella* sp. strain HA-1 in the small-sized conical HTP *(18)*. Moreover, the high photosynthetic productivity of the tested strain observed even in the presence of CO_2, NO, and SO_2 is likely indicative of the capacity of the *C. sorokiniana* strain HO-1 to adapt to the stack gases emitted by thermal power plants.

A maximum photosynthetic productivity of 10.5 g of carbon/(m^2·d) has been obtained with a marine chlorophyte, *Tetraselmis suecica*, in shallow outdoor flumes, which is commonly used as a culture system for commercial production, under full sunlight in Hawaii *(16)*. This value corresponds to a photosynthetic efficiency of 4.71% (PAR) for the light energy input of 2540 kcal/(m^2·d) (This photosynthetic efficiency value was calculated based on the data in the papers *[16,19]*.). Recently, the photosynthetic efficiencies obtained with various outdoor *S. platensis* cultivation systems in Florence, Italy, during the summer were summarized by Tredici and Zittelli *(20)*. They reported photosynthetic efficiencies of 6.6% (PAR) in a coiled tubular reactor, 4.6% (PAR) in a sun-oriented plate, and 6.0% (PAR) in a vertical plate. Compared with these values, the photosynthetic efficiency of 8.67% (PAR) that we obtained using the *C. sorokiniana* strain HO-1 and our conical HTP under an average photosynthetic photon flux density simulating the outdoors in central Japan was a quite high value. The photosynthetic efficiency of 6.87% (PAR), simulating the strong, midday summer sun (light energy input of 16,702 kJ/[m^2·d]), was also a high value. The green microalga *C. sorokiniana* strain HO-1 attained high photosynthetic performance even at high temperature and high light intensity in the conical HTP.

Thus, the capacity of the *C. sorokiniana* strain HO-1 to tolerate high CO_2, NO, and SO_2; high temperatures; and high light intensities is of obvious benefit for its use in outdoor cultures in the midsummer and makes this strain a good candidate for use in a CO_2 fixation/conversion system aimed at utilizing a certain amount of CO_2 from thermal power plants.

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