COLLECTION OF HIGH ENERGY YIELDING STRAINS OF SALINE MICROALGAE FROM THE HAWAIIAN ISLANDS

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Richard H. York, Jr.
Hawaii Institute of Marine Biology
46-007 Lilipuna Road
Kaneohe, Hawaii 96744-1346

ABSTRACT

Microalgae were collected from 48 locations in the Hawaiian Islands in 1985. The sites were an aquaculture tank; a coral reef; bays; a geothermal steam vent; Hawaiian fish ponds; a Hawaiian salt punawai (well); the ocean; river mouths; saline lakes; saline pools; saline ponds; a saline swamp; and the ponds, drainage ditches and sumps of commercial shrimp farms. Conductivities of the water ranged from 6.00×10^2 micromhos cm^{-1} to 3.85×10^5 micromhos cm^{-1}, and temperatures ranged from 10.5°C to 62.0°C. Single cells or colonies of microalgae were isolated into media in glass culture tubes incubated in fluorescent light in the laboratory, and into fluorocarbon plastic bags transmitting full-spectrum sunlight outdoors. From 4,800 isolations, 100 of the most productive clones were selected to be maintained by periodic transfer to sterile medium. Five clones were tested for growth rate and production in a full-spectrum-transmitting solarium. The cultures were bubbled with carbon dioxide and air. Temperatures ranged from 17.7°C to 42.8°C. The highest growth rates were 2.12 doublings day^{-1} for *Chaetoceros sp.* clone SH 9-1, and 1.43 doublings day^{-1} for *Cyclotella sp.* clone 14-89. The highest production was 31 g dry weight m^{-2} day^{-1} for the *Chaetoceros sp.*, and 33 g dry weight m^{-2} day^{-1} for the *Cyclotella.*

PROJECT OBJECTIVES

Task I: Make collections from sites in the Hawaiian Islands that would favor desired characteristics and select for strains that are either dominant at the time of collection or become dominant in enriched media at high light.

Task II: Selection of the most productive strains.

Task III: Screen at least five of the most promising strains for growth rate and productivity.

METHODS

Task I

The collection sites were photographed, and the date, time, name and coordinates noted. At most sites, a vertically integrated sample was collected by lowering an open bottle. The depths sampled, and the maximum depth of the water body were recorded. Dissolved oxygen concentration (DO) and pH were measured electrochemically *in situ* if the water was deep enough to cover the electrodes. Temperature was measured *in situ* with a mercury thermometer. Water collected for the isolation of cells was sub-sampled for determination of the concentrations of algal nutrients (N, P, Si), chlorophyll, and microalgal cells. Nutrient samples were filtered through pre-combusted Whatman GF/F filters and frozen until analyzed with a Technicon autoanalyzer. Chlorophyll samples were collected on GF/F filters and stored in methanol in a freezer until analyzed in a Turner fluorometer. Unfiltered water was preserved by the addition of Lugol's solution for estimation of the population densities of microalgal cells using a Spiers-Levy Eosinophil counting chamber and compound microscope. Other sub-samples of water were sterile-filtered for measurement of electrical conductivity, and for use as one type of culture medium.

Culture medium was prepared with 4 types of sterile-filtered water: 1) sample water; 2) offshore seawater adjusted to the conductivity of the sample by dilution with distilled water or concentrated by evaporation; 3) SERI Type I water, 40 millimhos cm^{-1}

or the conductivity nearest to that of the sample; and 4) SERI Type II water, 40 millimhos cm^{-1} or the conductivity nearest to that of the sample. These were enriched to make half-strength f medium (f/2) including 107 micromolar sodium metasilicate (Guillard and Ryther, 1962). Each type of medium was pipetted to two types of culture vessels: 1) Aclar plastic bags, and 2) glass culture tubes.

For each collection 100 single cells or colonies of microalgae were isolated using the modified Pasteur-type pipette technique (Guillard 1973; Hoshaw and Rosowski 1973) with a stereomicroscope, and each placed in individual bags or tubes containing 10 ml of medium.

Unfiltered sample water was enriched to make f/2 medium without added silicate, and 10 ml each was pipetted to Aclar bags and glass culture tubes. Another tube and bag received culture after silicate was added. If the sample was sparsely populated, microalgae were concentrated on filters which were also placed in a bag and tube of enrichment water including silicate. The Aclar bags were placed outdoors in direct sunlight without temperature control. The culture tubes were placed in the laboratory and illuminated 16 hours per day by Vita-Lite fluorescent lights, at temperatures of 25°C to 26°C.

One large-scale outdoor culture was made by pumping 1,700 l of seawater into a 5.5-m diameter fabricated pool and enriching it to make f/2 medium including 107 micromolar silicate. Isolations were made after microalgal growth was evident.

Another method used was to collect sediment from a dried pond bottom. This sample was then placed in a petri dish in the laboratory, hyrated with distilled water, and incubated under the fluorescent lights.

Task II

The densest cultures were examined microscopically for condition of the cells and for contamination. Clones selected were transferred to 40 ml f medium in 125 ml Erlenmeyer flasks with cotton plugs, incubated under the same conditions as the culture tubes and transferred to new medium periodically. If contamination was observed, cultures were recloned or treated with antibiotics. Clones being maintained in brackish water or water

of conductivity higher than seawater were also inoculated into seawater medium. If growth occurred, subsequent transfers were made into seawater medium.

Task III

A full-spectrum-transmitting solarium was designed and constructed by covering aluminum framing with 0.127 mm thick Aclar type 22A plastic film. An air-conditioner was adjusted to produce air temperatures less than 20°C at night and greater than 30°C during the day. Air temperatures were measured with a maximum-minimum indicating mercury thermometer. Incident irradiance was measured with a Weathertronics silicon cell pyranometer, model 3120.

Culture temperatures were measured at intervals with a mercury thermometer to find that maxima, minima, and relationship to the solarium air temperature at different culture densities and irradiances. Growth rate was determined by measuring optical density at a wavelength of 750 nannometers (OD 750) using a Beckman DU-7 spectrophotometer. When the OD 750 exceeded 0.1, the values for the cultures were calculated from measurements of dilutions. Production was measured as dry weight at 60°C, and ash-free dry weight from combustion at 450°C.

Culture vessels were constructed by heat-sealing Aclar plastic film to make bags. Culture medium was prepared by enriching sterile-filtered seawater to make f medium including sodium metasilicate for Bacillariophytes (diatoms). One liter of medium was added to each bag. Aclar bags were inoculated with one of 5 clones to densities near 0.01 OD 750. The clones were *Chaetoceros sp.* clone SH 9-1, *Cyclotella sp.* clone SH 14-89, *Platychloris sp.* clone SH 6-22, a Cyanophyte clone SH 14-22, and a Pyrrophyte clone SH 22-20. As the cultures became dense, more nutrients were added to prevent nutrient limitation. The cultures were bubbled with a mixture of carbon dioxide and air.

RESULTS AND DISCUSSION

Task I

Microalgae were collected from 48 locations in the Hawaiian Islands during the year 1985. The sites were an aquaculture tank; a coral reef; bays; a geothermal steam vent; Hawaiian fish ponds; a Hawaiian salt punawai (well); the ocean; river mouths; saline lakes; saline pools; saline ponds; a saline swamp; and the ponds, drainage ditches and sumps of commercial shrimp farms. Table 1 lists the sites and their locations.

Table 2 lists the collection site data. Conductivities ranged from 6.00×10^2 micromhos cm^{-1} for water collected from an algal film growing on rock in a geothermal steam vent, to 3.85×10^5 micromhos cm^{-1} for dried pond bottom sediment after it was hydrated with distilled water, resulting in the growth of the flagellate *Dunaliella*. The lowest temperature recorded was 10.5°C in an abalone/kelp culture tank which was receiving ocean water from a depth of 600 m through the experimental Ocean Thermal Energy Conversion plant at Keahole Point. There was a bloom of *Skeletonema* in this tank. The highest temperature was 62.0°C in the middle of clumps of viable algal film in the geothermal steam vent. The lowest dissolved oxygen concentration was 0.6 ppm in the drainage ditch at Mariculture Research and Training Center (MRTC). The drainage ditch had an organic film on the surface, an odor of hydrogen sulfide, and a dark reddish-brown coloration from suspended iron precipitate and organics. Viable *Nitzchia* cells were isolated from this unfavorable environment. The highest dissolved oxygen concentration was 18.4 ppm in pond number 108 at the Amorient shrimp farm. This was three times the saturation concentration. *Chaetoceros* sp. cells were isolated from the pond. The most supersaturated dissolved oxygen concentration was in the punawai (salt well) sample at Salt Pond on Kauai. The water was so salty that the 8.6 ppm DO was 3.7 times saturation. There was a thick bloom of *Dunaliella sp.* forming a surface film that entrapped large oxygen bubbles.

Successful cultures were obtained from all 48 collection sites. The success rate was higher in the laboratory than outdoors. This was probably due to photoinhibition and higher temperatures outdoors. Clones that were tolerant of the conditions outdoors are desirable for large-scale culture. Some clones, including *Chaetoceros, Cyclotella,*

Table 1. Collection locations

Collection Number	Name	Location	Island	Latitude North	Longitude West
1	Kalakaua's Fishpond	Kahaluu	Hawaii	19°34'53.8"	155°58'11.2"
2	Abalone/kelp tank	Keahole	Hawaii	19°43'54.8"	156°03'41.3"
3	Pacific Ocean	Kalae	Hawaii	18°54'48.7"	155°41'00.4"
4	Geothermal steam vent	Kamaili	Hawaii	19°26'36.4"	154°56'41.9"
5	Hilo Bay	Mokaoku	Hawaii	19°43'58.8"	155°03'59.8"
6	Kahana Pond	Kahalui	Maui	20°53'33.0"	156°27'28.0"
7	Salt Pond	Hanapepe	Kauai	21°54'10.6"	159°36'33.6"
8	Kealia Pond	Kealia	Maui	20°47'45.4"	156°28'48.0"
9	Aquatic Farms, Pond 9	Hakipuu	Oahu	20°30'33.7"	157°51'23.5"
10	Honokowai Stream	Honokowai	Maui	20°57'12.3"	156°41'34.2"
11	Kulanihakoi Stream	Kalepolepo	Maui	20°46'08.7"	156°27'37.0"
12	Kealia Pond	Kealia	Maui	20°47'49.0"	156°28'30.8"
13	Kealia Pond	Kealia	Maui	20°48'00.6"	156°29'22.5"
14	Menehune Fishpond	Huleia	Kauai	21°57'05.4"	159°22'39.6"
15	Nualolo Kai Reef	Nualolo Kai	Kauai	22°09'46.0"	159°42'11.4"
16	Kilauea River	Kilauea	Kauai	22°13'27.3"	159°23'23.2"
17	Wailua River	Wailua	Kauai	21°57'22.6"	159°40'02.4"
18	Salt Lake	Salt Lake	Oahu	21°21'27.8"	157°54'39.2"
19	Apua Fishpond	Kualoa	Oahu	21°30'46.8"	157°50'23.5"
20	Molii Fishpond	Kualoa	Oahu	21°30'39.0"	157°50'52.2"
21	Kaneohe Bay	Moku o loe	Oahu	21°26'11.2"	157°47'19.3"
22	Amorient, Pond 106	Kahuku	Oahu	21°41'01.6"	157°57'59.0"
23	Amorient, Pond 107	Kahuku	Oahu	21°41'01.6"	157°58'00.0"
24	Amorient, Pond 108	Kahuku	Oahu	21°41'01.6"	157°58'01.0"

Table 1. Collection locations
(continued)

Collection Number	Name	Location	Island	Latitude North	Longitude West
25	Amorient, Pond 138	Kahuku	Oahu	21°41'03.0"	157°58'02.9"
26	Amorient, Pond 139	Kahuku	Oahu	21°41'03.0"	157°58'04.1"
27	Amorient, Pond 140	Kahuku	Oahu	21°41'03.0"	157°58'05.8"
28	Amorient, Pond 141	Kahuku	Oahu	21°41'03.0"	157°58'06.6"
29	Amorient, Pond 142	Kahuku	Oahu	21°41'03.0"	157°58'07.9"
30	Amorient, Pond 143	Kahuku	Oahu	21°41'03.0"	157°58'08.7"
31	Amorient, drainage ditch	Kahuku	Oahu	21°41'03.4"	157°58'00.4"
32	Amorient, sump	Kahuku	Oahu	21°41'04.4"	157°58'00.0"
33	Enchanted Lake	Kailua	Oahu	21°23'03.5"	157°44'00.8"
34	Kupeke Fishpond	Kupeke	Molokai	21°04'38.0"	156°47'41.7"
35	Orca Seafarms, drainage ditch	Kahanui	Molokai	21°06'	157°05'
36	Manawainui Swamp	Kahanui	Molokai	21°06'	157°05'
37	Orca Seafarms, Pond GP7	Kahanui	Molokai	21°06'	157°05'
38	Orca Seafarms, Pond GN6	Kahanui	Molokai	21°06'	157°05'
39	Marine Research & Training Center (MRTC), Pond 2	Hakipuu	Oahu	21°30'	157°51'
40	MRTC, Pond 3	Hakipuu	Oahu	21°30'	157°51'
41	MRTC, Pond 4	Hakipuu	Oahu	21°30'	157°51'
42	MRTC, Pond 5	Hakipuu	Oahu	21°30'	157°51'
43	MRTC, Pond 7	Hakipuu	Oahu	21°30'	157°51'
44	MRTC, Pond 8	Hakipuu	Oahu	21°30'	157°51'
45	MRTC, Pond 10	Hakipuu	Oahu	21°30'	157°51'
46	MRTC, Pond 11	Hakipuu	Oahu	21°30'	157°51'
47	MRTC, Pond 12	Hakipuu	Oahu	21°30'	157°51'
48	MRTC, drainage ditch	Hakipuu	Oahu	21°30'	157°51'

Table 2. Collection site data

Collection Number	Date	Time	Conductivity (micromhos cm^{-1})	Depth of Sample (cm)	Maximum Water Depth (cm)	Dissolved Oxygen (ppm)	pH	Temperature (^{o}C)
1	05/13/85	10:30	5.90×10^{3}	0 - 10	100	6.9	7.1	24.8
2	05/13/85	15:00	4.40×10^{4}	50	400	-	-	10.5
3	05/16/85	16:00	4.50×10^{4}	0 - 100	1×10^{6}	5.8	8.2	25.0
4	05/17/85	16:00	6.00×10^{2}	epilithic	0	-	-	62.0
5	05/18/85	12:15	3.13×10^{4}	0 - 50	1,300	6.1	8.2	23.0
6	05/29/85	16:00	2.96×10^{4}	0 - 5	20	8.6	9.2	29.0
7	05/31/85	18:50	2.20×10^{5}	0 - 10	200	8.6	8.2	29.0
8	05/29/85	15:00	3.85×10^{5}	------dry sediment------		-	-	-
9	07/10/85	15:30	3.27×10^{4}	0 - 100	100	12.8	7.4	31.5
10	07/10/85	15:30	8.90×10^{3}	0 - 30	100	12.8	7.4	31.5
11	07/14/85	15:13	3.62×10^{4}	0 - 10	100	7.3	8.2	31.5
12	07/15/85	10:21	1.87×10^{4}	0 - 1	1	5.7	8.7	33.0
13	07/16/85	09:55	5.00×10^{4}	0 - 30	100	5.5	8.3	27.2
14	08/07/85	17:10	1.78×10^{4}	0 - 20	200	5.8	7.5	29.2
15	08/09/85	12:30	4.50×10^{4}	0 - 100	1,000	7.4	8.2	28.0
16	08/10/85	18:00	3.42×10^{4}	200	400	6.6	8.2	29.0
16A	08/10/85	18:00	2.88×10^{3}	10	400	8.0	7.3	29.5
17	08/11/85	11:00	2.83×10^{4}	50	200	5.8	8.2	28.0
18	08/28/85	15:35	2.28×10^{4}	0 - 15	100	6.9	8.2	31.0
19	09/09/85	11:00	5.14×10^{4}	0 - 5	10	4.4	8.8	37.2
20	09/09/85	12:20	4.90×10^{4}	10	200	5.7	8.2	30.5
21	09/23/85	20:30	4.60×10^{4}	200	1,600	10.9	8.2	27.8
22	10/07/85	13:20	1.93×10^{4}	0 - 100	100	9.4	8.5	28.0
23	10/07/85	13:45	2.12×10^{4}	0 - 10	10	18.4	9.8	33.0
24	10/07/85	14:15	2.80×10^{4}	0 - 60	60	8.0	9.8	33.0

Table 2. Collection site data
(continued)

Collection Number	Date	Time	Conductivity (micromhos cm^{-1})	Depth of Sample (cm)	Maximum Water Depth (cm)	Dissolved Oxygen (ppm)	pH	Temperature (^{o}C)
25	10/07/85	14:25	5.75×10^{4}	0 - 10	10	6.4	8.3	31.0
26	10/21/85	09:30	1.10×10^{4}	0 - 30	30	9.9	8.6	25.5
27	10/21/85	09:45	1.48×10^{4}	0 - 100	100	7.9	8.2	25.5
28	10/21/85	10:05	1.72×10^{4}	0 - 100	100	7.5	8.0	25.5
29	10/21/85	10:15	1.76×10^{4}	0 - 100	100	8.8	8.1	25.5
30	10/21/85	10:30	1.13×10^{4}	0 - 50	50	8.8	8.3	26.0
31	10/21/85	10:45	4.60×10^{3}	0 - 100	100	4.2	7.8	26.0
32	10/21/85	10:55	8.19×10^{3}	0 - 100	100	10.4	8.1	26.8
33	11/04/85	14:40	2.24×10^{4}	0 - 10	122	4.2	6.8	27.0
34	11/07/85	12:00	3.33×10^{4}	0 - 20	200	9.6	-	28.0
35	11/20/85	13.25	3.73×10^{4}	0 - 10	100	6.5	-	28.0
36	11/20/85	14:00	4.46×10^{4}	0 - 10	10	15.0	-	35.0
37	11/20/85	15:00	2.42×10^{4}	0 - 20	20	13.0	-	34.0
38	11/20/85	15:15	3.78×10^{4}	0 - 20	20	11.2	-	35.0
39	12/04/85	11:40	2.43×10^{4}	0 - 12	12	9.8	-	25.0
40	12/04/85	12:03	1.86×10^{4}	0 - 25	25	9.4	-	25.0
41	12/04/85	12:15	1.89×10^{4}	0 - 100	100	16.4	-	25.0
42	12/04/85	12:25	2.28×10^{4}	0 - 100	100	11.6	-	25.0
43	12/04/85	12:40	1.82×10^{4}	0 - 50	50	15.8	-	26.0
44	12/04/85	12:50	1.79×10^{4}	0 - 100	100	10.2	-	25.0
45	12/04/85	13:00	2.63×10^{4}	0 - 100	100	14.4	-	25.0
46	12/04/85	13:15	2.23×10^{4}	0 - 50	80	10.8	-	26.0
47	12/04/85	13:20	2.82×10^{4}	0 - 50	100	9.4	-	25.0
48	12/04/85	13:30	2.02×10^{4}	0 - 10	34	0.6	-	26.8

and *Melosira* grew rapidly from a single cell to high densities at temperatures up to 42°C with full-spectrum solar irradiance.

In some cases, species that grew well as clones did not bloom in the enrichment of the unfiltered water sample from which they were isolated. This may have been the result of competition, disease or predation.

There was not much difference in the success rate of clones from the same population in the media of different water types.

The 1,700-l pool enrichment yielded a flocculant bloom composed mostly of pennate diatoms which tolerated temperatures as high as 42.2°C and direct sunlight. These were successfully isolated.

Task II

A total of 100 clones were selected to be maintained by periodic transfer to sterile medium. Most are being grown in seawater medium. The clones that have been identified are Bacillariophytes, Chlorophytes, Cyanophytes and Pyrrophytes.

Isolates that grew successully in the Aclar bags were selected. Pennate diatoms from the pool enrichment were also selected because they had bloomed in high light intensities and temperatures. Clones from the *Nitzschia* population at Nualolo Kai were selected because of the large lipid droplets observed in the cells in the sample. This was probably the result of low nutrient concentrations in the seawater.

The clones of *Melosira* that were selected formed long chains that could easily be harvested by conventional screen-type harvesters used in *Spirulina* culture. For this reason, these clones may have lower production costs than other microalgae in large-scale cultures.

Task III

Of the five clones tested only the *Chaetoceros sp.* clone SH 9-1 and *Cyclotella sp.* clone SH 14-89 grew. The *Platychloris* and the Pyrrophyte were still motile, but were probably inhibited by temperatures ranging from 17.7°C to 42.8°C, or were photoinhibited, or both. These may also have been the reasons that the Cyanophyte did not grow. The highest solar irradiance recorded at noon was 960 watts m^{-2}.

Nutrients were added on Days 7, 8 and 11. The cultures were diluted on Day 15. Growth of the *Chaetoceros* and the *Cyclotella* is shown in Figures 1 and 2. Growth rates are listed in Table 3. The highest growth rates were 2.12 doublings day^{-1} for the *Chaetoceros*, and 1.43 doublings day^{-1} for the *Cyclotella*. The highest production was 31 g dry weight m^{-2} day^{-1} for the *Chaetoceros*, and 33 g dry weight m^{-2} day^{-1} for the *Cyclotella*.

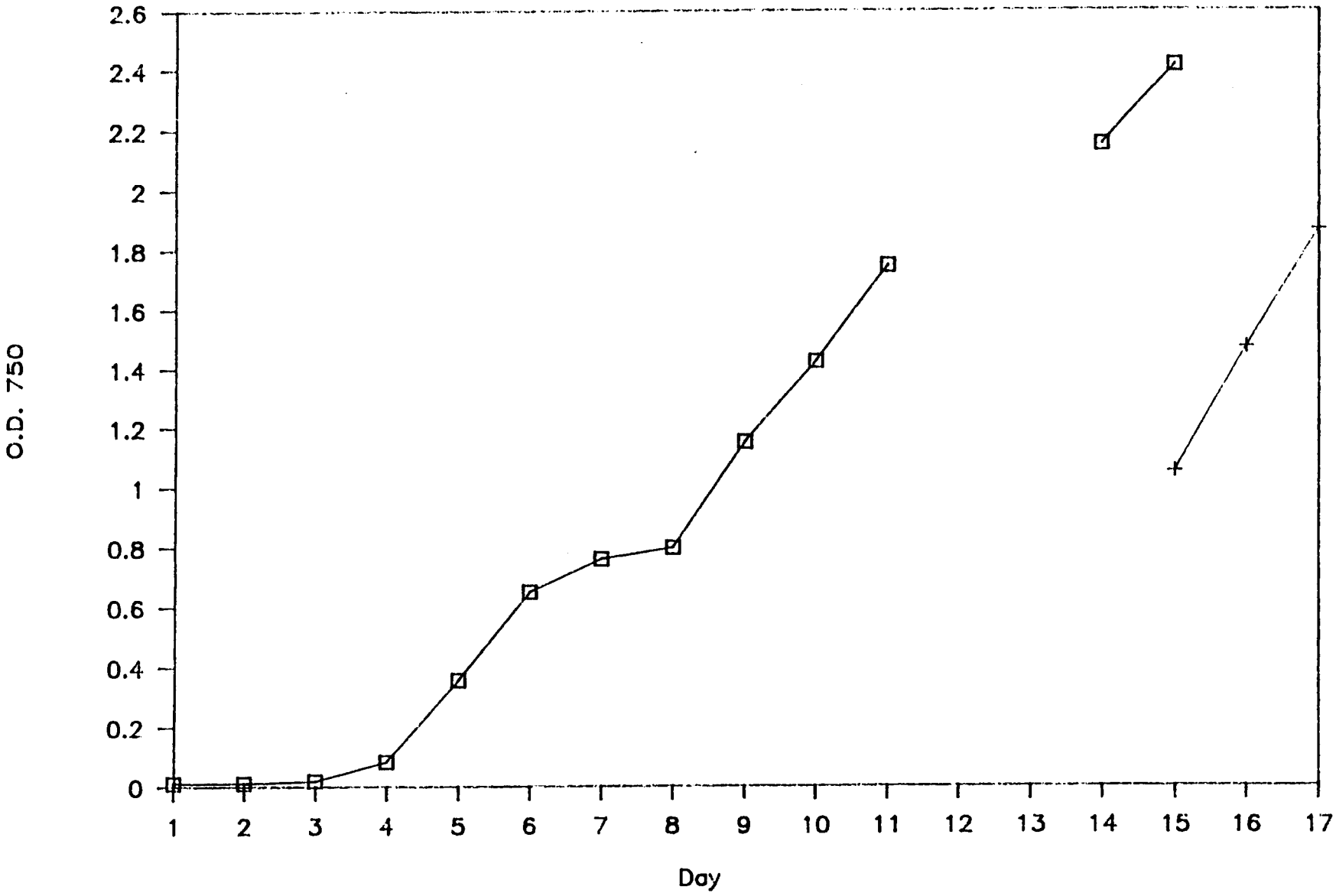

Figure 1. Growth of Chaetoceros sp. clone SH 9-1

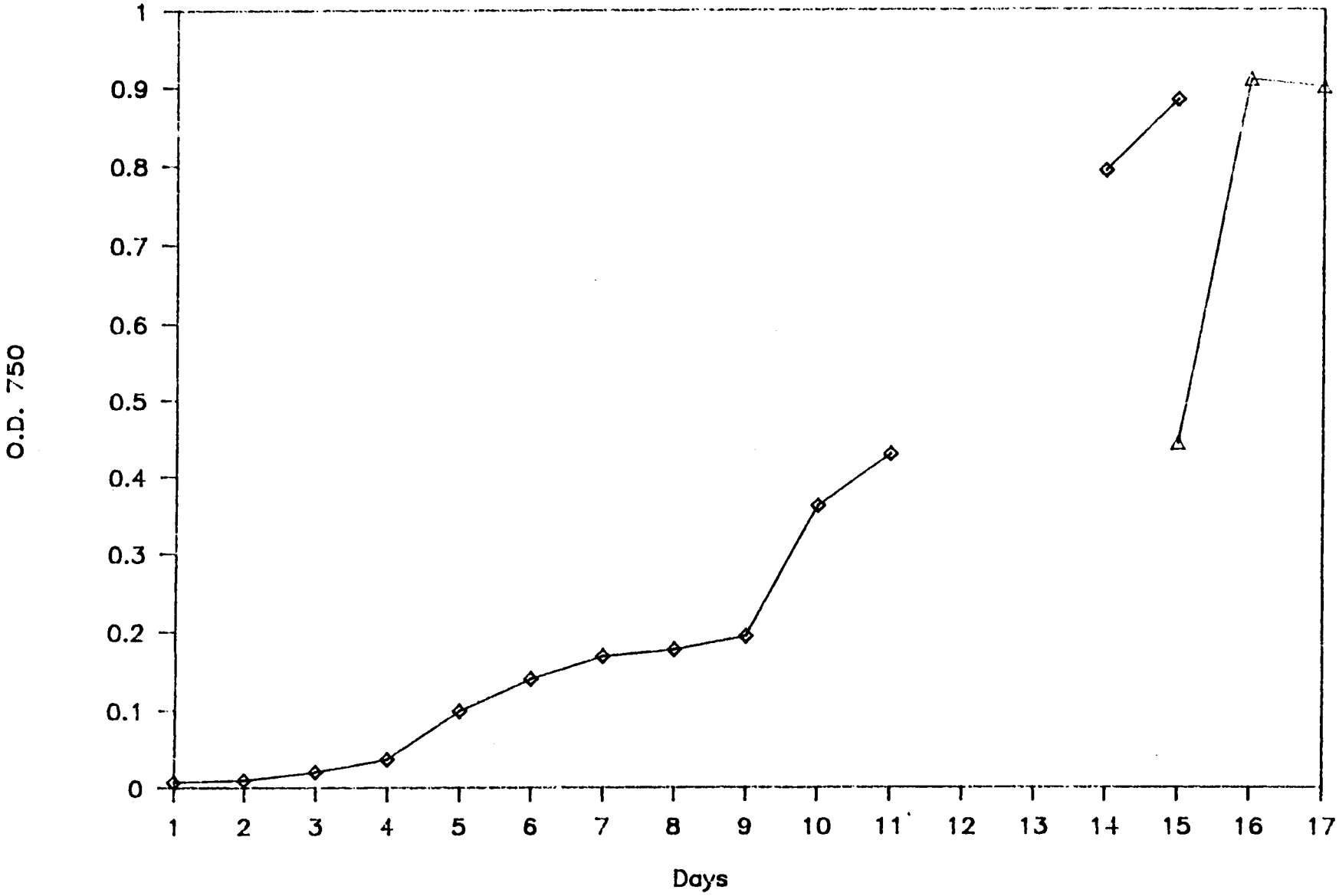

Figure 2. Growth of Cyclotella sp. clone SH 14-89

Table 3. Growth rates of Chaetoceros and Cyclotella clones
(doublings per day)

Day	Chaetoceros	Cyclotella
2	0.01	0.39
3	0.78	1.06
4	2.12	0.86
5	2.09	1.43
6	0.86	0.50
7	0.23	0.27
8	0.07	0.07
9	0.53	0.13
10	0.31	0.90
11	0.53	0.25
14	0.30	0.88
15	0.17	0.15
16	0.48	1.04
17	0.33	0

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