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Growth Kinetics of *Cylindrotheca closterium* (Ehrenberg) Reimann and Lewin Isolated from Aegean Sea Coastal Water (Izmir Bay/Türkiye)

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Abstract: The aim of this research was to reveal the detailed information on growth kinetics of *Cylindrotheca closterium* (Ehrenberg) Remann and Lewin from the results of nutrient enrichment experiments under batch culture conditions. *C. closterium* was isolated from Izmir Bay. The present study were performed under optimum light and nutrient saturated conditions except the nutrient, which was investigated. An exponential growth rate was obtained by using each growth curves. The exponential growth rates were plotted against corresponding each nutrient concentrations. The parameters of Monod curve were calculated with the least square method by using transformations to linear form. Half Saturation Constant (Ks) was calculated for only silicate as 9.58 μM maximum growth rate were found as 4.32 day^{-1}.

Key words: *Cylindrotheca closterium,* growth rate , silicate, nutrient enrichment

INTRODUCTION

Phytoplankton is the base of pelagic food webs and the community composition of phytoplankton is important for the functional structure of the aquatic ecosystem[1].

Benthic micro-algae (Microphytobenthos) are quantitatively very important components from inter-tidal mudflats to continental shelf systems[2,3].

Microphytobenthos (constituted mainly by diatoms)[4] is the major food supply for numerous inter-tidal species. Thus, it is obvious that microflora might play an important role in accumulation of contaminants through the coastal food chains[5] .

Epipelic (mud dwelling c.f.)[6] diatoms are the dominant microphytobenthos in many inter-tidal, soft-sediment habitats[7-9]. Microphytobenthic biofilms exhibit high rates of primary production (up to 300 g C m^{-2} y^{-1})[10,11] can contribute up to 50% of estuarine primary production[11]. Epipelic diatoms are motile and migrate through the sediment in response to tidal and diurnal rhythms[9,12-14], appearing at the surface of sediments during periods of emersion and migrating into the sediment when the sediment becomes immersed[5].

Specific maximum growth rate (μ_{max}) and Half Saturation Constant (K_m or K_s) are only applicable to single species and are unique physiological characteristics of a species , not a community. Even small changes in species composition or changes in dominance of species during an algal bloom will have a major influence on these physiological parameters[15].

Nutrient enrichment incubation is an operational tool with which to examine potential nutrient limitation, in which of water to determine if algal growth is stimulated[16].

Such studies are important arguments to describe trophic-level dynamics in food chains of systems.

C. closterium is widely distributed in mudflat assemblages and can be used as model organism in understanding the role diatoms play in mudflat habitats.

Motility and aggregation of mudflat diatom *Cylindrothoca closterium (Bacillariophyceae)* under varying environmental conditions[17].

C. closterium (*Nitzschia closterum*) produces DMSP (Dimethylsulphoniopropionate)[18,19] and it is often abundant in intertidal sediments[20].

Yet species-specific response will enable us to predict, to some extent, which species will dominate depending on environmental conditions[21].

It will lead to a better understanding and prediction of responses of lower trophic level in Middle and Inner Parts of Izmir Bay which exposures with treated waste waters.

Here, this study present and discuss nutrient enrichment experiments on *Cylindrotheca closterium* isolated from Inner Part of Izmir Bay.

MATERIALS AND METHODS

Study area: The Bay of Izmir is located in the Western part of Turkey and surrounded by a densely populated community. The Bay is divided into a Inner, Middle and

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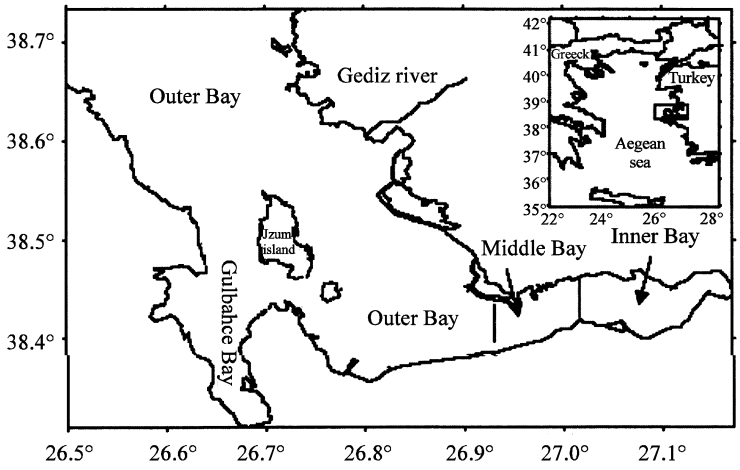

Fig. 1: Map of Izmir Bay

Outer Bay from the standpoint of topographical and hydrographical characteristics. Inner Bay is small in area (57 km^2) and shallow in depth (max. 15 m). It receives the majority of domestic and industrial waste waters (Fig. 1). This part of the Bay also receives some inflow of fresh waters from several creeks which are mostly polluted by industrial wastes. Because of limited water exchange with the Outer Bay and Aegean Sea pollution of the Inner Bay has reached unacceptable levels. Eutrophication of the Inner and the Middle Bay is spreading progressively to the Outer Part of the Bay. Red-tide occurrence is reported to have increase in frequency in recent years. For this reason Izmir Municipality decided to construct Izmir Big Channel Waste Water Project in 1969. But unfortunately Water treatment Construction could not be completed until now. In 2000-January half of the water treatment plant opened and 65% of the sewage water started to treat until January 2000. This is why, the pollutant levels of the Inner Bay water decreased slowly.

Microalgal culture: For this study *C. closterium* was isolated from Izmir Bay (Aegean sea). At the time of the study, cultures of *C. closterium* were unialgal but non-axenic. *C. closterium* cultures were grown in 2l Erlenmayer flasks containing 1.5 l of sterile f/2 medium[22].

Experiments were conducted in a constant temperature room at 18°C and irradiated at 1300 ft Cd (footcandle) by daylight fluorescent lamps. Cultures were maintained on a 12:12 light:dark cycle.

Before the laboratory experiments started, the sea water was filtered using a Sartorius filtering cardriges capsules (0.45+0.2 μ pore size).

Enrichment experiments: For batch culture experiments, f/2 and h/2 mediums were used by Guillard[23]. For the experiment, the concentrations of nutrients in f/2 medium were changed and thus, for every nutrient a different concentration has been obtained.

The experiments were carried out in 1 L Pyrex bottles initially containing 1 L of seawater.

The final concentrations were obtained by adding the ambient levels to the exposured concentrations of every single experiment.

Initial chlorophyll a concentrations at the start of each experiment groups were 1 μg L^{-1}.

Experimental bottles were gently shaken on a daily basis, to keep the cells in solution and to keep *C. closterium* cells for adhering to the bottle wall.

Analytical methods: Ammonium, phosphate and silicate concentrations were analysed according to Strickland and Parsons[24] and nitrate by the copper-cadmium reduction method of UNESCO[25]; these analyses were carried out by using Bosch-Lomb Spectronic 21UVD model spectrometer. Also *in vivo* chlorophyll a concentrations were measured by using TURNER 10-AU model Fluorometer.

Specific growth rates: Chlorophyll a specific growth rates were calculated from chlorophyll a concentrations during experimental growth. The specific growth rate was obtained from each growth curve calculating the following equation[26]:

$$\mu = \frac{3.322^* \log(N_2/N_1)}{\Delta t}$$

Where, N_2 and N_1 and the chlorophyll a concentration at the end (t_2) and the beginning (t_1) of a period of time, Δt is the $t_2 - t_1$.

The growth rate has been shown by Monod[27] to be related to the concentration of substrate medium by the equation: $\mu = \mu_{max}(S/ K_s+S)$.

Where, μ is the specific growth rate μ_{max} is the maximum growth rate unlimited by low concentrations of the substrate, S, K_s (Half-Saturation Constant) is the concentration that supports a rate equal to $\mu_{max}/2$.

RESULTS AND DISCUSSION

According to analytical results, natural seawater concentrations are NO_3^--N: 3.67 μM, NH_4^+-N: 160.7 μM, PO_4^{-3}-P: 4.2 μM, $Si(OH)_4$: 7.30 μM.

Because of the very high background concentrations of ammonium (160.7 μM), growth curves of *C. closterium* did not show significant differences (Fig. 2).

Growth of *C. closterium* increased within 24 h upon NH_4^+-N. Cultures entered directly logaritmic phase then entered a phase of slower growth, whose duration varied with the nutrient treatment: the stationary phase started on day 3 in 160.7 and 170.7 μM NH_4^+-N treatment. Addition of 260.7 μM ammonium, *C. closterium* cultures entered directly death phase after the exponential phase.

Specific growth rates was found as 0.82, 0.8 and 2.11 day^{-1} for 161.7 μM, 170.7 μM and 260.7 μM NH_4^+-N treatment, respectively.

At <200 μM ammonium concentrations specific growth rate is around 1 day^{-1} same as that of the phosphate at <5 μM. The higher values than 200 μM caused to have growth rate of population increase but this increment inconsistent with Michealis-Menten Curve (Fig. 3).

After addition of different concentrations of phosphate to the experiment bottles, 5 different growth curves obtained (Fig. 4). For 4.20, 4.23 and 4.5 μM phosphate concentrations, cells entered directly to the exponential phase and lasted about 4 days and passed through the transition phase and stayed only one day then entered the death phase. This similarity depends on the nutrient concentrations which were not quite different from each other.

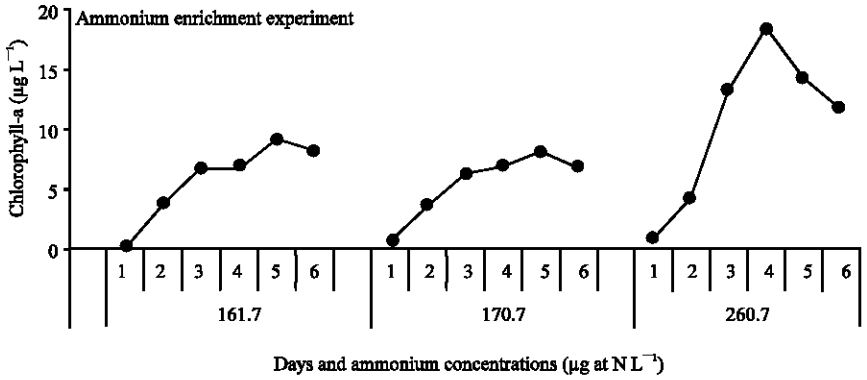

Fig. 2: Growth curves of different concentrations of ammonium. Each curves are the ammonium concentrations (initial+added) in the medium, as μmol L^{-1}

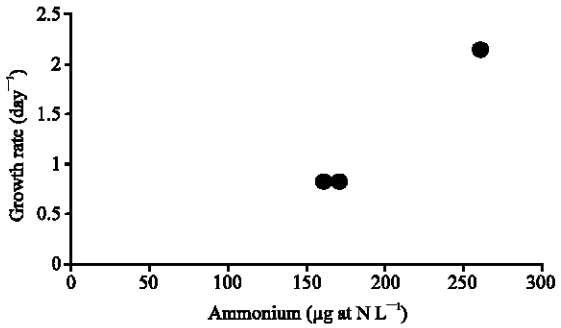

Fig. 3: Plot of growth rates against ammonium concentrations

Table 1: The specific growth rates calculated from growth curves which obtained from different nutrient additions to *C. closterium* cultures

Exposured nutrient concentrations for ammonium (μM)	161.7	170.7	260.7		
Specific growth rates (μ)	0.82	0.80	2.11		
Exposured nutrient concentrations for phosphate (μM)	4.20	4.23	4.50	6.01	7.89
Specific growth rates (μ)	1	1	1	0.79	0.79
Exposured nutrient concentrations for nitrate (μM)	3.67	12.15	47.87		
Specific growth rates (μ)	3.06	2.03	2.12		
Exposured nutrient concentrations for silicate (μM)	7.39	8.39	17.39	34.12	60.82
Specific growth rates (μ)	2.27	2.34	2.66	2.82	3.99

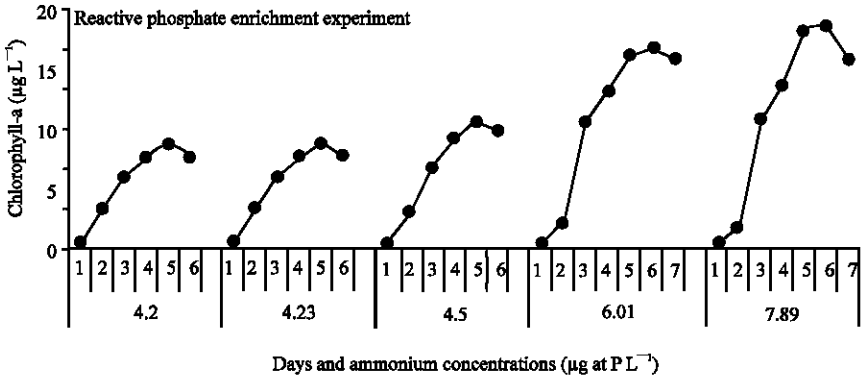

Fig. 4: Growth curves of different concentrations of phosphate. Each curves are the phosphate concentrations (initial+added) in the medium, as μmol L^{-1}

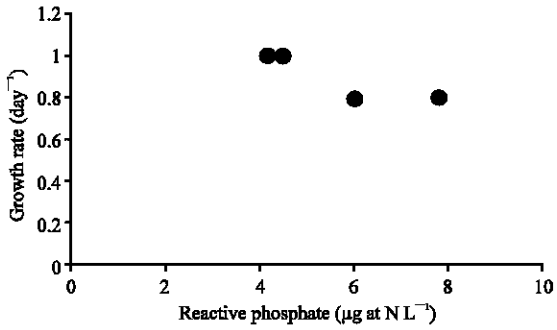

Fig. 5: Plot of growth rates against phosphate concentrations

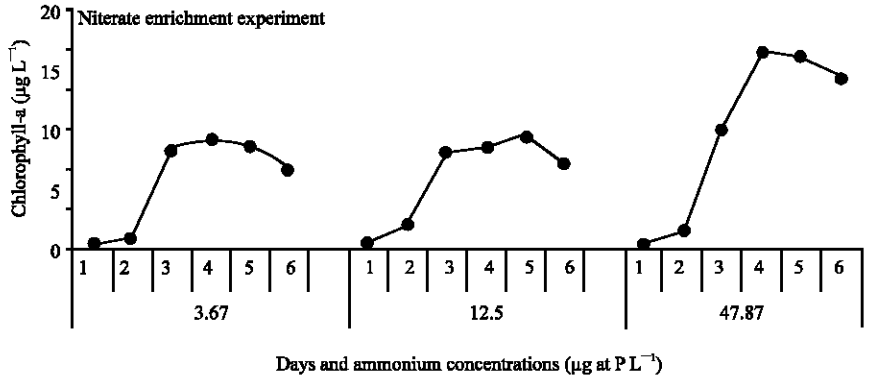

Fig. 6: Growth curves of different concentrations of nitrate. Each curves are the nitrate concentrations (initial+added) in the medium, as μmol L^{-1}

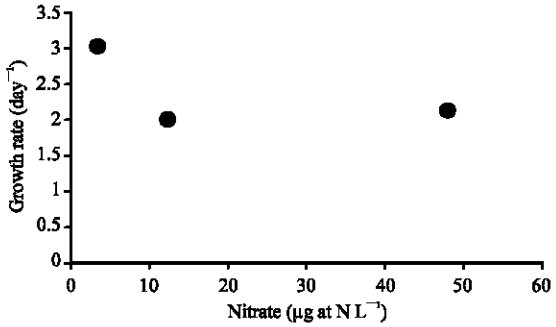

Fig. 7: Plot of growth rates against nitrate concentrations

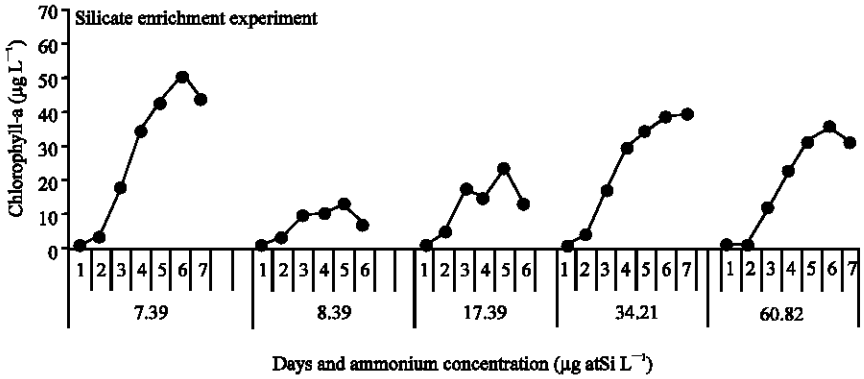

Fig. 8: Growth curves of different concentrations of silicate. Each curves are the silicate concentrations (initial+added) in the medium, as µmol L^{-1}

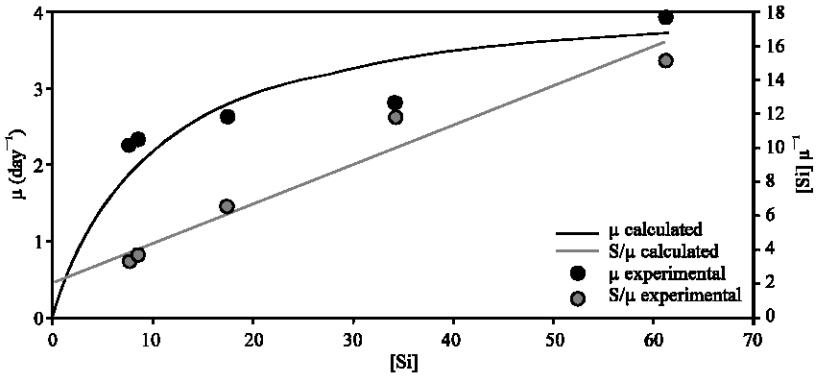

Fig. 9: Specific growth rates as a function of silicate concentration. The S/µ versus S regression line was used to calculate K_s and μ_{max}. Black circles: /µ versus S, gray circles: S/µ versus S

Specific growth rates was found as 1 day^{-1}, for 4.20, 4.23 and 4.50 µM PO_4^{-3}-P treatment. For 6.01 and 7.89 µM PO_4^{-3}-P treatment, specific growth rates calculated as 0.79 day^{-1} (Fig. 5).

Reactive phosphate levels which lower from 5 µM, specific growth rates was calculated 1 day^{-1} (Table 1).

Table 1 shows the specific growth rates calculated from growth curves which obtained from different nutrient additions to *C. closterium* cultures.

At nitrate enrichment experiment groups 3 different nitrate concentrations had been exposured for observing growth curves of *C. closterium*. Growth curves has been presented in Fig. 6.

As it can be seen in Fig. 7 >10 µM Nitrate and >5 µM phosphate concentrations exhibited growth inhibition. Ambient levels of these nutrients didn't limit the growth.

In this research, it was calculated the highest specific growth rates for silicate experiment groups.

Specific growth rates was found as 2.27 day^{-1}, 2.34 day^{-1}, 2.66 day^{-1} 2.82 day^{-1} and 3.99 day^{-1} for 7.39 μM, 8.39 μM, 17.39 μM , 34.12 μM and 60.82 μM silicate treatment, respectively (Fig. 8).

For *C. closterium*, at 18°C constant temperature, 1300 ft Cd light intensity and by adding different silicate concentrations, Half-Saturation Constant (K_s) value was found out as 9.58 μM and the maximum growth rate as 4.32 day^{-1} (Fig. 9).

In situ growth rate was calculated from Michalis-Menten equation using 3.22 μM Si/L ambient silicate concentration. We understand from this value that growth rate is reached to 25% of maximum growth rate at this *in situ* silicate concentration. Silicate is strongly limiting nutrient for this experiments.

Whatever nutrient levels are high in marine water sampling environment, open Aegean sea waters which are entered in to the Bay by currents have an oligotrophic characteristics. So that we found very similar K_s values which are reported by Mangin *et al.*[28] for open sea waters.

Silicate limited waters which are discharged from waste water treatment plant, go out to the Izmir bay with water circulation system. Because of this reason the most important silicate resources of Izmir Bay are deep waters (sediment/water interactions) and non-point terrestrial resources during rainfall period.

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