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Effect of physicochemical conditions on growth rates of cyanobacteria species isolated from Lake Magadi, a soda lake in Kenya

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Abstract

Cyanobacteria contribute significantly to primary productivity by adding nitrogen into the ecosystem through nitrogen fixation. They are also used as biofertilizers, food and feed while others produce compounds that have potential biotechnological application. Samples of water were collected from the shoreline of Lake Magadi in January 2012 and isolation of cyanobacteria species that live in the hypersaline conditions of the lake carried out. The isolation was done on Aiba and Ogawa medium for halophilic cyanobacteria and identification of the isolates was based on cell and colony morphology. The effect of physicochemical factors such as salinity, pH and temperature, on the growth rate of the isolates was also investigated. Colony forming, unicellular and filamentous species were isolated. The isolates were found to exhibit tolerance to environmental extremes and hence, the ability to survive in the hypersaline and halophilic conditions of the soda lake. The data obtained may be useful to environmentalists when there is need to vary primary productivity of aquatic ecosystems.

Key words: Soda lake, cyanobacteria, cell and colony morphology, physicochemical conditions.

INTRODUCTION

Lake Magadi is located about 2° S, 36° 20´E, the elevation above sea level is about 600 m and it covers an area of 90 km^2. It has salinity of up to 30% w/v, pH values of about 11.5 and water temperatures frequently above 40°C (Duckworth et al., 1996).

It is largely a dry bed that fills up with water only after rains when water enters the northern part of the lake through temporary watercourses. Shallow lagoons in the northern and southern ends of the lake are fed by hot springs all year round. The lake water is saturated with CO_2 and the molar concentration of HCO_3^- and CO_3^{2-} ions greatly exceeds that of Ca^{2+} and Mg^{2+}.

As a result of evaporation, saturation of these alkaline earth cations is rapidly achieved and they precipitate out of solution as insoluble carbonates. This leaves Na^+, Cl^-, HCO^{3-} and CO^{3-} ions as the major ions in solution (Jones et al., 1998).

The lake is however, a productive ecosystem due to the availability of dissolved carbon dioxide, high ambient temperatures and high daily light intensities (Melack and Kilham, 1974) and harbors diverse species of microorganisms. These include hetrotrophic cyanobacteria which can inhabit almost every conceivable environment, from oceans to fresh water and bare rock to soil.

They are the only group of organisms able to reduce nitrogen in aerobic conditions, a fact that may be responsible for their evolutionary and ecological success. The ability of cyanobacteria to perform oxygenic photosynthesis is thought to have converted the early reducing atmosphere into an oxidizing one, which dramatically changed the composition of life forms on earth by stimulating biodiversity and leading to the near-extinction of oxygen-intolerant organisms (Schultz, 2009).

Cyanobacteria account for 20 to 30% of the Earth's photosynthetic productivity and exert an extensive effect on the dynamics of carbon flow in many of the marine environments (Martin and Kokjohn, 1999) as well as other aquatic ecosystems. They also produce a variety of compounds that have shown potential application in major disease management, such as cancer, asthma, arthritis, diabetes and HIV (Skulberg, 2000).

They have also shown immense potential in wastewater and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, biofertilizers, fuel and cosmetics (Fatma, 1999). Species such as *Arthrospira* constitutes the only food source for the vast flocks of lesser flamingo (*Phoeniconaias minor*) that graze on some African soda lakes. Some species such as *Aphanizomenon flos-aquae* and *Arthrospira platensis* are used as food (Spolaore et al, 2006).

Researchers have also shown the potential of generation of “Clean and Green Energy” through converting sunlight directly into electricity (Jacobson, 2009).

Heterocyst-forming species such as *Anabaena* are specialized for nitrogen fixation and are able to fix atmospheric nitrogen into ammonia (NH_3), nitrites (NO_2^-) or nitrates (NO_3^-) which can be absorbed by plants. Due to their ability to fix nitrogen in aerobic conditions, they are often found as symbionts with a number of other groups of organisms such as fungi (lichens), corals and pteridophytes (Azolla) and angiosperms (Gunnera) (Enrique-Flores, 2008).

When cyanobacteria blooms occur, irradiance is reduced leading to reduction in the growth of producers that cannot maintain a position near the surface of the water, including epiphyton, benthic algae and rooted vascular plants. Thus, lakes with very dense blooms, especially if they are frequent or long lasting, may not support large populations of other producers (Scheffer et al., 1993).

Intense blooms with high photosynthetic activity also deplete free CO_2 from lake water. This may stimulate formation of surface scums and dominance by cyanobacteria taxa that can move to the air-water interface where CO_2 is available, shading other algae in the process (Paerl and Ustach, 1982).

There is evidence that high pH during intense cyanobacteria blooms may be toxic to certain species of fish (Kann and Smith, 1999). Oxygen depletion that occurs in the water during bloom senescence can also lead to the death of organisms in the water. There is also the impact of high levels of ammonia during bloom senescence that can cause mortality of snails and other macro invertebrates (Jones, 1987). Certain cyanobacteria such as *Microcystis aeruginosa* produce cyanotoxins which can be neurotoxins, hepatotoxins, cytotoxins, and endotoxins, and can be toxic and dangerous to humans and animals.

A common observation is that cyanobacteria dominance of aquatic communities is greater when water temperatures are warmer (Brock, 1975). Waterbury et al. (1979) outlined the factors controlling the abundance of cyanobacteria species and included grazing, viral mortality, genetic variability, light adaptation, temperature as well as nutrients.

In 1953, 1959 and 1962, *Alcolapia Grahami,* a fish species that feeds on algae, was introduced to Lake Magadi in order to feed on mosquitoes. By the 70s, the fish species had become one of the main consumers of algae which were primarily the food for lesser flamingo. In response to the presence of the fish, populations of other birds such as white pelican increased.

In 1974, a report by ILEC (2001) indicated that the algae *Spirulina platensis* had disappeared from the lake for reasons that remained unknown, leading to a reduction of algal blooms, primary productivity and resident number of flamingoes (http//www.ilec.or.jp/en/pubs/p2/lake-resvr). The decline in cyanobacteria population may have led to the massive die-off of fish reported by Wetlands International (http//www.wwt.org.uk) that occurred in 1991. Cyanobacteria therefore, play an important role in maintaining the stability of Lake Magadi ecosystem.

The purpose of this study was to investigate the effect of some physicochemical conditions on the growth rates and abundance of cyanobacteria species. This was done by isolating and characterizing species by morphology and then, subjecting the isolates to growth in varying conditions of temperature, pH and salinity.

The data obtained could be used by environmentalists when making management decisions regarding soda lakes and keeping track of species that could be put to use by biotechnologists.

MATERIALS AND METHODS

Study site and sampling of water

Lake Magadi is situated in a closed lake basin about 100 km south of Nairobi and has a depth that does not exceed 1 m. Samples of water were taken randomly from sites with different water coloration ranging from colorless, bluish and brownish to green. Sampling was done at the shores of the lake in the month of January, 2012. About 300 ml of sample water was collected from each site in three sterile 150 ml culture bottles. The samples were transported in a cool box to the Laboratory and kept at 4°C awaiting analysis.

Isolation of cyanobacteria

Aiba and Ogawa medium for marine cyanobacteria was prepared as two separate solutions to avoid precipitation:

- Solution A consisted of 2.0 g/L NaCl, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 2.0 g/L K_2SO_4, 80 mg/L $CaCl_2 \cdot 2H_2O$, 5 g/L $NaNO_3$, 20 mg/L $FeSO_4 \cdot 7H_2O$ and 2.0 ml/L Gaffron micronutrients.
- Solution B consisted of 1.0 g/L K_2HPO_4, 8.06 g/L Na_2CO_3, and 27.22 g/L $NaHCO_3$.

After autoclaving, solution A was mixed with solution B aseptically

Table 1. Growth characteristics of extremophiles were used to classify the isolates (Wiegel, 1998).

Growth characteristics	Minimum	Optimum	Maximum
Halotolerant	-	$[Na^+]$ < 0.2 m	$[Na^+]$ > 0.2 m
Halophilic	$[Na^+]$ ≥ 0.2 m	$[Na^+]$ 0.2-1.7 m	-
Extreme halophile	$[Na^+]$ ≥ 0.2 m	$[Na^+]$ ≥ 1.7 m	-
Alkali tolerant	≥ pH 6	< pH 8.5	> pH 9.0
Facultative alkaliphilic	< pH 7.5	≥ pH 8.5	-
Obligate alkaliphilic	≥ pH 7.5	≥ pH 8.5	≥ pH 10.0
Thermotolerant	-	< 50°C	> 50°C
Thermophilic	-	50°C	55°C

after cooling to about 60°C giving a final pH of 9.4 to 9.8 (http:www-cyanosite.bio.purdue.edu/media/table/AO.html).

To build up the population of the microbes, the water samples were enriched with Aiba and Ogawa liquid media in the ratio of 1: 1 and incubated at room temperature in (23 ± 2°C) next to a window allowing enough sunlight for 10 days.

The cyanobacteria suspension obtained was subcultured by the spread plate technique using plates of Aiba and Ogawa media containing 15 g/L of agar. Lawns of cyanobacteria were observed under the dissecting microscope and each discrete colony was picked using a sterile inoculating needle and deposited into fresh liquid media in culture bottles. The cultures were left to grow for ten days under sunlight. This process of subculturing in solid media and then in liquid media was repeated until pure cultures were obtained.

Images of lawns and colonies of cyanobacteria isolates were taken by digital camera (Sony Cyber-Shot, DSC 5730 model) and by low power optical microscope respectively. Isolates were identified by their cell size and shape, cell motility and colony morphology.

Determination of growth rate of cyanobacterial isolates

Fifty milliliters of Aiba and Ogawa media was inoculated with each pure isolate using sterile inoculating needle. This was replicated thrice. The cultures were incubated at room temperature around (23 ± 2°C) next to a window allowing enough sunlight, for over two weeks. Growth rate was determined by counting the number of cells of each unicellular isolate using the Neubauer chamber after every two days.

Characterization of cyanobacterial isolates

Determination of optimum temperature for growth

Five milliliters of each of the cyanobacteria isolates broth culture was added to 45 ml of freshly prepared Aiba and Ogawa nutrient broth. The cultures were incubated at the following temperatures: room temperature (23 ± 2°C), 30, 40, 50 and 60°C next to a window allowing enough sunlight. This temperature range included the optimal temperature for enzyme-controlled metabolism (25 to 40°C) and the optimal temperature for thermophiles (50 to 60°C). (Table 1). The length of daylight does not vary substantially in Kenya, staying within 12 h and 12 min throughout the year. Light is therefore not likely to be a limiting factor for growth particularly in the months of January to March when the sun is around the Equator.

The optical density of each broth culture was measured using a spectrophotometer (Beckmann Coulter Du 530 model) at wavelength of 686 nm (Brown, 2005) at incubation and after every two days for 14 days. The cultures at each temperature were replicated thrice. Analysis of variance (ANOVA) and F-test were used to test for statistically significant differences (Payne et al., 2009)

Determination of optimum salt concentration for growth

Aiba and Ogawa medium was prepared with different NaCl concentrations (w/v) of 0, 0.1, 0.3, 0.6 and 0.9%. This range of salinities used was to determine the halophilic or halotolerant nature of the isolates. (Table 1). Five milliliters of each broth culture was added to 45 ml of the liquid medium with different salt concentrations and incubated at room temperature (23 ± 2°C) next to a window allowing sunlight for 14 days. The optical density of each broth culture was measured at the beginning and after every two days. The cultures at each salinity level were replicated thrice.

Determination of optimum pH for growth

Using concentrated hydrochloric acid and universal paper strips to monitor pH, 200 ml of broth media for halophilic cyanobacteria had the pH adjusted from 10 to 7 and 6. This range of pH conditions were chosen to determine the alkaliphilic or alkalitolerant nature of the isolates. (Table 1). The media were filter sterilized using a of 0.22 µm pore size membrane filter. Forty- five milliliters was poured into sterile culture flasks and inoculated with 5 ml stock broth cultures of each isolate. The cultures were incubated at room temperature (23 ± 2°C) next to a window allowing enough sunlight and optical densities measured at the beginning and after every two days for two weeks. The cultures at each pH condition were replicated thrice.

RESULTS

Cell and colony morphology of cyanobacteria isolates

Seven isolates with distinct morphology were obtained (Table 2). Unicellular colonial and filamentous colonial cyanoabacteria were isolated from Lake Magadi. Cell sizes ranged from about 0.5 to about 2 µm. Three isolates were motile while others were not. Two isolates had unique colonies (one had colonies that segregated

Table 2. Morphology of cyanobacteria species isolated from Lake Magadi.

Isolate	Morphology	Reproduction	Cell and colony structure
MCy1	Unicellular and colonial	Binary fission	Cells with cylindrical shape with a groove in the middle, about 1.5 µm in length, undulating motility, remain loosely attached after cell division, broth culture appears dark green
MCy2	Unicellular and colonial	Binary fission	Spherical cells with dark streaks, about 2 µm in diameter, dark colonies under the optical microscope, yellowish green liquid cultures
MCy3	Unicellular and colonial	Binary fission	Cells are spherical, about 1 µm in diameter, gliding motion, small rounded colonies
MCy4	Unicellular and colonial	Binary fission	Cells are tiny, less than 1 µm and spherical
MCy5	Filamentous and colonial	Fragmentation of trichome	Coiled filaments, spirals are not as close as in MCy6, heterocytes present, colonies float on the surface of liquid medium
MCy6	Filamentous and colonial	Growth of chain of cells	Closely spiraling filaments
MCy7	Filamentous and colonial	Growth of chain of cells	Straight filaments forming a membranous sheet of colonies that floats at the bottom of the liquid medium, oscillation movement

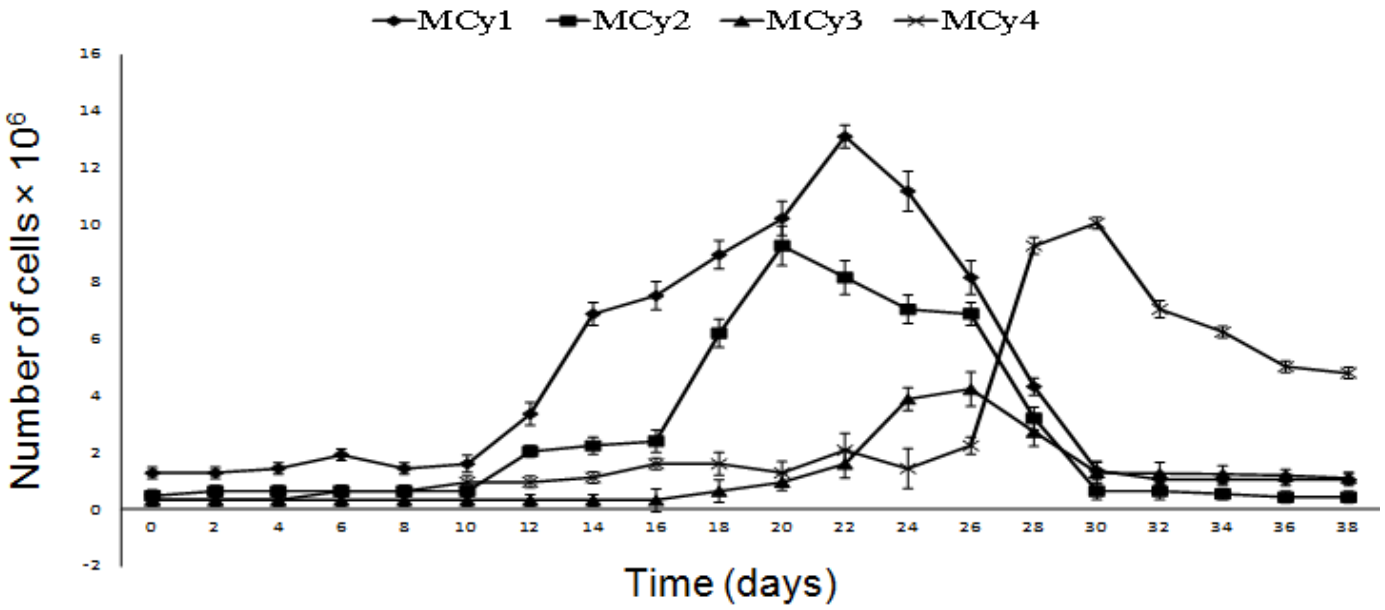

Figure 1. Population (number of cells/ml) of unicellular cyanobacteria species isolated from water sampled from Lake Magadi.

after 10 days of growth while the other had small, discrete colonies) that could be used for identification of the species. The mode of motility was also used for identification. (Table 2).

Growth curves of cyanobacteria isolates

Growth curves of four unicellular cyanobacteria species isolated from Lake Magadi showed lag phases ranging from 12 days with isolate MCy1 to 28 days with isolate MCy4 (Figure 1). Isolate MCy3 had the least population growth with a maximum of about 4×10^6 cells/ml while isolate MCy1 had a maximum of about 1.4×10^7 cells/ml.

Effect of temperature on growth rate of cyanoacteria isolates

Growth rate of cyanobacteria incubated at 50°C (without light) and at room temperature (23 ± 2°C) next to a window allowing plenty of sunlight increased with time. Growth rate in the incubator at 60, 40 and 30°C respectively, declined with time for most of the isolates (Figure 2). At 50°C, the cells had less chlorophyll which was indicated by a much lighter shade of green and there was less aggregation of cells into colonies. The measurement of optical density of isolate MCy7 was difficult as it formed membrane like masses of colonies that were difficult to homogenize.

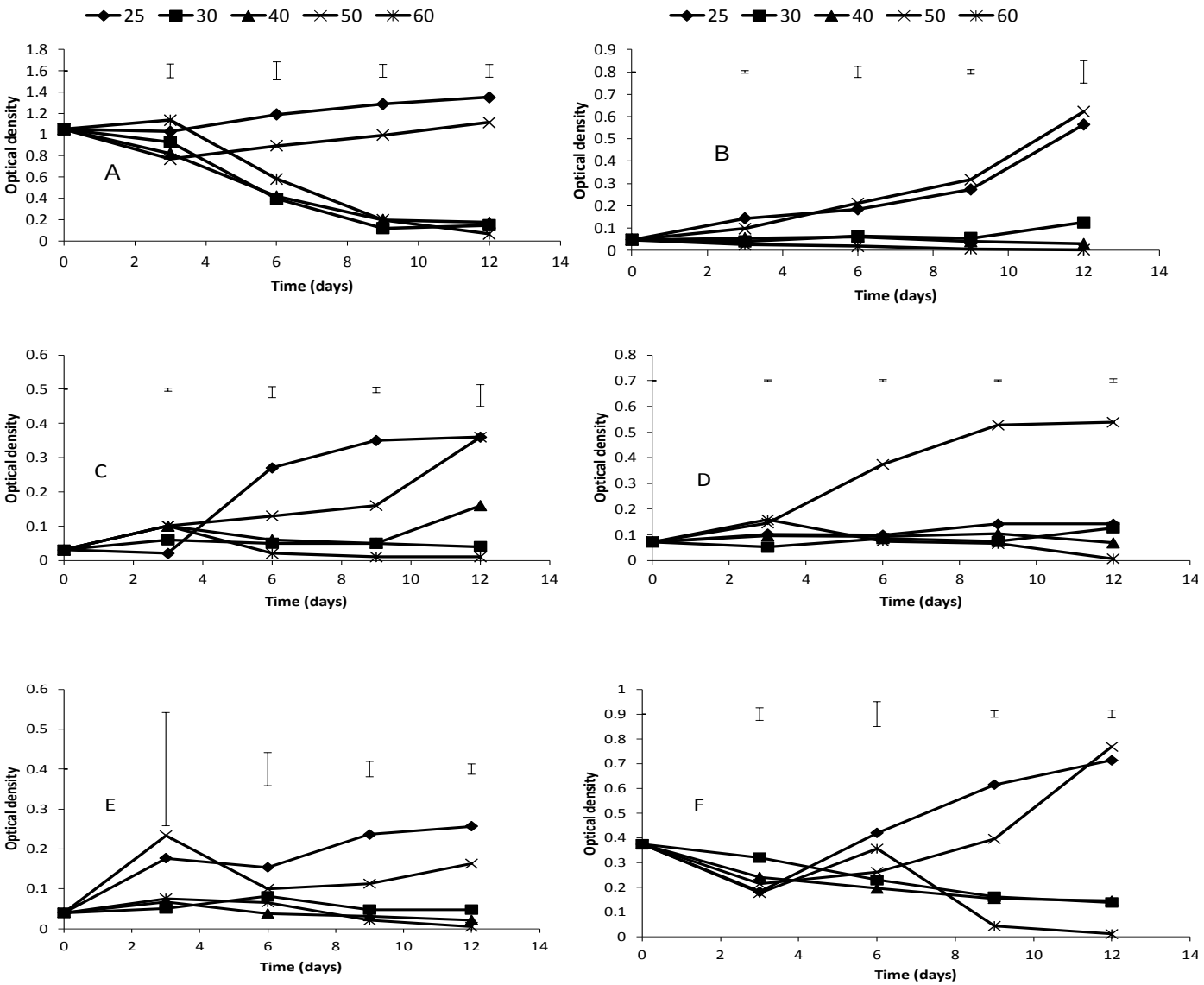

Figure 2. Growth rates of cyanobacteria species isolated from samples of water from Lake Magadi (measured as optical density) at varying temperature.

Temperature had a significant ($p<0.001$) effect on the growth rates of isolate MCy1, MCy2, MCy4, MCy5 and MCy6 but it did not have a significant ($p=0.471$) effect on the growth rate of isolate MCy3 (Figure 2C).

The highest growth rate was at 25°C with means of optical density going up to 1.181 followed by 50°C. The lowest growth rate was at 60, 40 and 30°C respectively with the highest mean being 0.5282. The three temperatures did not have significant differences in growth rates between them.

Effect of pH on growth rate of cyanobacteria isolates

Growth rate of cyanobacteria isolated from samples of water from Lake Magadi was seen to be higher at pH 6 for four of the isolates (Figure 3A, C, D and E). Two of the isolates had higher growth at pH 7 (Figure 3B and F) though, they appeared to grow relatively well in all the pH conditions. The pH value had a significant ($p<0.001$) effect on growth rates of cyanobacteria isolate MCy1, MCy3, MCy4 and MCy5 but did not have a significant ($p=0.377$) effect on growth rate of isolate MCy2. The highest growth rate was at pH 6 with a mean of 0.8158 while pH 7 had the least mean of 0.6138.

Effect of salinity on growth rate of cyanobacterial isolates

Growth of cyanobacteria species isolated from Lake Magadi increased with time. Salinity had a significant

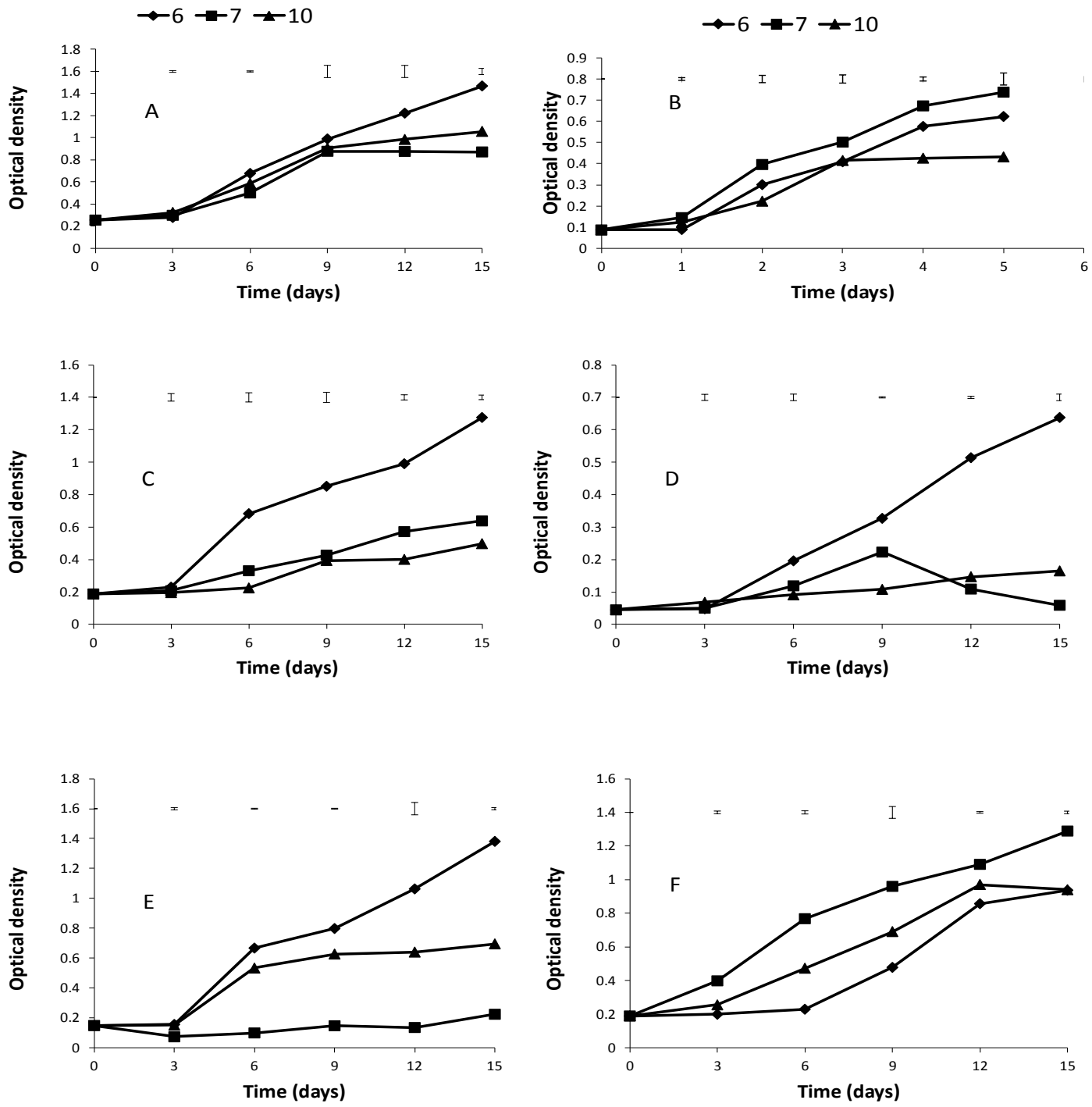

Figure 3. Growth rates of cyanobacteria species isolated from samples of water from Lake Magadi (measured as optical density) in varying pH conditions.

(p=0.008) effect on the growth of isolate MCy1 where salinity of 0.6% w/v had the highest mean (79.72) while other salinities were not significantly different from each other. Salinity had a significant (p<0.001) effect on growth rates of isolates MCy1, MCy2, MCy4, MCy5 and MCy6 but did not have a significant (p=0.423) effect on the growth rate of isolate MCy3 (Figure 4C). Salinity of 0.0% w/v had the highest means for isolates MCy4, MCy5 and MCy6.

DISCUSSION

Cells of isolate MCy1 were similar to cells of *Synechococcus*, a unicellular cyanobacterium whose size varies from 0.8 to 1.5 µm that prefers well-lit surface

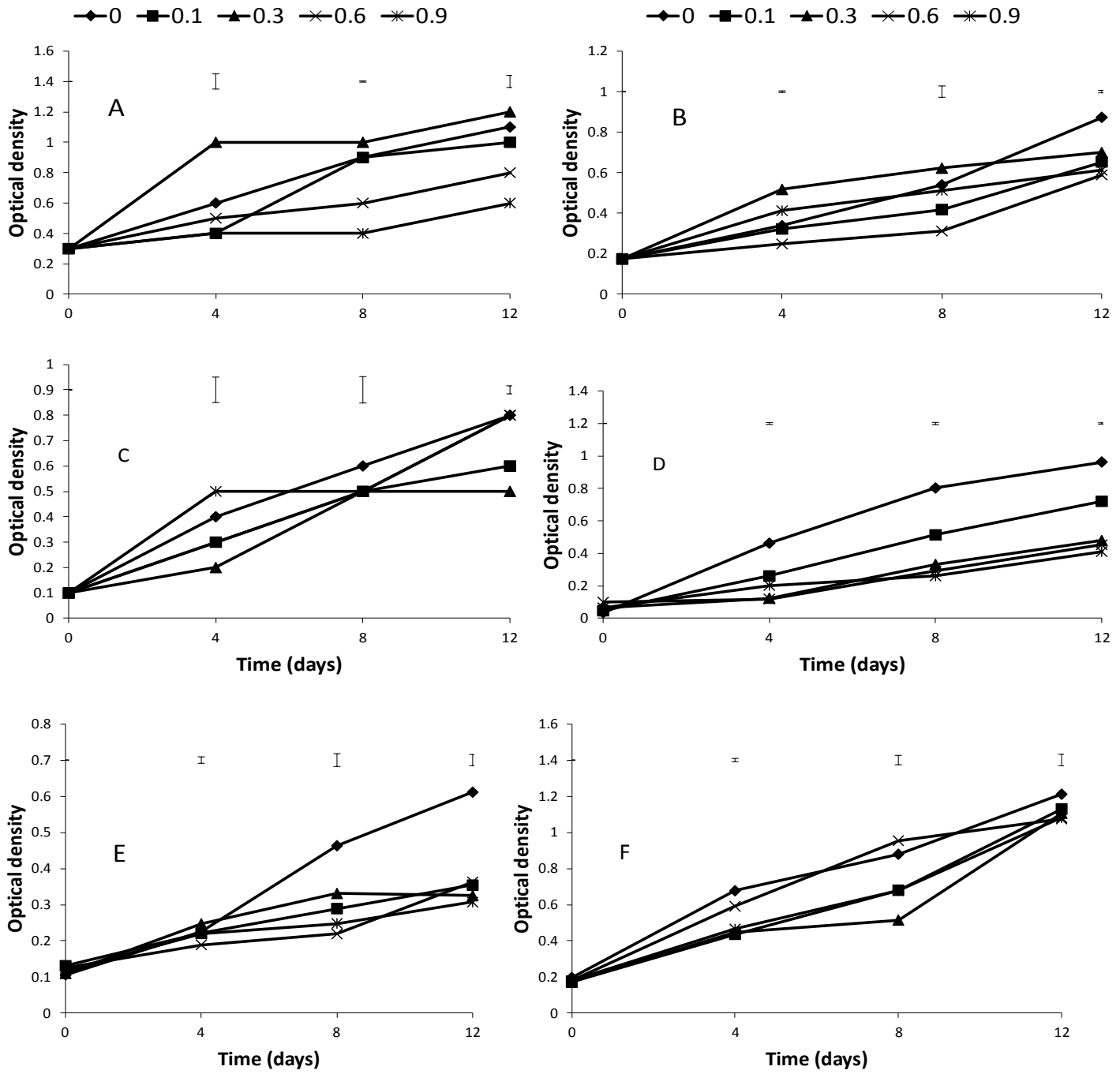

Figure 4. Effect of salinity on growth rates (measured as optical density) of cyanobacteria species isolated from water samples from Lake Magadi.

waters. Cells are known to be motile by a gliding type method and a novel uncharacterized, non-phototactic swimming method that does not involve flagella motion.

Isolate MCy2 was similar to *Microcystis*, a genus of freshwater cyanobacteria characterized by small cells of about 2.5 to 5.5 µm in diameter. The cells are usually organized into colonies that begin in a spherical shape, but losing their coherence to become perforated or irregularly shaped over time. The coloration of the protoplast is a light blue-green, appearing dark or brown due to optical effects of gas-filled vesicles which are useful as a distinguishing characteristic when using light microscopy (Lalita et al., 2009).

Isolate MCy3 had unicellular cells of about 1 µm that were spherical, exhibited gliding motion and formed small discrete colonies characteristic of *Synechocystis*. The growth rate of the species appeared to be limited by factors other than temperature and salinity. It has a

photosynthetic apparatus very similar to the one found in plants and exhibits phototactic movement (Anderson and McIntosh, 1991).

Among all cyanobacteria species, *Synechocystis* sp., PCC 6803 is one of the most extensively studied species since it was initially isolated from a freshwater lake in 1968. The entire genome, including four endogenous plasmids, was sequenced, and over 3000 genes have been annotated to date (Kaneko et al., 2003).

Synechocystis 6803 demonstrates versatile carbon metabolisms, growing under photoautotrophic, mixotrophic and heterotrophic conditions (Vermas, 1996). Additionally, biochemical similarities between the plant chloroplasts and *Synechocystis* 6803 make the latter an ideal system for studying the molecular mechanisms underlying stress responses and stress adaptation in higher plants (Los, 2010).

Isolate MCy4 with minute cells of about 0.5 µm similar to *Prochlorococcus*, the smallest known photosynthetic organism whose cell size varies from 0.45 to 0.75 µm (Lindell, 1998) possesses a pigment complement, which includes divinyl derivatives of chlorophyll (Chl a and b*)*, which are referred to as Chl a2 and b2 that are unique to this genus (Neveux, 1993).

One of the most intriguing ecological characteristics of *Prochlorococcus*, besides its capacity to grow over a very wide range of irradiances in nature, is its ability to colonize extremely oligotrophic areas. Under these conditions, the small cell size and the resulting high surface-to-volume ratio are adaptative advantages for nutrient uptake (Chisholm, 1992).

The heterocystous filaments of isolate MCy5 that formed a slime layer at the surface of the medium were similar to those of *Anabaena* which has gas vacuoles that inflate or deflate with air to provide upward or downward movement. This adaptation positions *Anabaena* at a favorable depth, determined by available sunlight, water temperature, or O_2 concentration. With optimal environmental conditions, *Anabaena* grows unchecked, forming large blooms on the surface of the water (Herrero and Flores, 2008).

Isolate MCy6 with closely twisted filaments was similar to *Arthrospira* (*Spirulina*) which is an oxygenic phototroph that can tolerate high levels of bicarbonate, carbonate, salinity and survives in water with a pH as high as 11. The species can reach very high cell densities and in laboratory cultures, the density has been recorded at 3 g dry weight per liter.

Isolate MCy7 had long unbranching filaments that were motile by oscillation motion and was identified as *Oscillatoria*. It uses the mobility to move towards light in order to conduct photosynthesis and forms a mass of colonies at the bottom of culture bottle. Källqvist (1981) investigated growth of *Planktothrix (Oscillatoria)* and found that it was able to grow at very low light intensities below 3 m depth. The ability of *Planktothrix* sp to grow at low light intensities and to harvest certain specific light qualities enables it to grow in the "shadow" of other phytoplankton.

Growth of cyanobacteria in culture media reaches maximum in about twenty four days after inoculation. All the isolates had optimal growth at room temperature (23 ± 2°C) when placed in plenty of sunlight next to a window and at 50°C in the incubator. Lack of light may have led to less amount of chlorophyll at 50°C but growth still continued. These findings were in agreement with those of Anderson and McIntosh (1991) that certain cyanobacteria species such as *Synechocystis* are capable of both phototrophic growth by oxygenic photosynthesis in sunlight and heterotrophic growth by glycolysis and oxidative phosphorylation during dark periods.

Many phytoplankton cellular processes are temperature dependent; their rates accelerating exponentially with increasing temperature, with maximal values occurring between 25 and 40°C (Reynolds, 1984). The photosynthetic and specific maximal growth rate responses of different species to temperature can be compared provided other factors, such as illumination and nutrients, remain saturating.

Responses are however, highly variable from one species to another (Reynolds, 1984). Co-existing communities of photosynthetic micro-organisms in lakes display both temporal and spatial patterns often associated with covariations in temperature (Tilman and Kiesling, 1984). Reynolds (1984) has pointed that apart from availability of nutrients, the other environmental factors controlling phytoplankton growth are temperature and light fluctuations.

Four isolates had higher growth rates at pH 6 while two had higher growth rates at pH 7. However, all isolates appeared to grow in all pH conditions tested. The growth rates of isolate MCy6 were relatively high in all pH conditions which agrees with studies on pH requirements for certain cyanobacteria species (Bano and Siddiqui, 2003) that showed *Spirulina major* to have high growth rates at all pH values, though, on the basis of chlorophyll content, best growth was obtained at pH 6.5, whereas highest growth was at pH 8.0 when protein content was taken into account.

Slightly acidic environment is not deterrent to the growth of cyanobacteria, and in some cases, it is the preferred pH for higher growth. Beside *S. major*, some other species were also able to grow in pH 6.5 but at lower rates and that there is a complete absence of cyanobacteria in the environment with pH less than 4 (Rippka et al., 1979).

Lower salinity of down to 0.0% w/v NaCl concentration favored the growth of all isolates while higher sodium chloride concentration of 0.6 to 0.9% w/v gave lower growth rates. These results are supported by studies by Kevin et al. (1987), done on a bloom of the cyanobacterium, *Microcyslis aeruginosa* in the upper Potomac River. It had densities of 193×10^6 cells 1^{-1}

and 83% of total cells in the surface mixed layer.

However, in regions typified by salinities of 1 to 2 ppt immediately down-river, the cyanobacteria disappeared from the phytoplankton assemblage, never contributing more than 17% of total phytoplankton numbers. Bloom samples collected from the river were exposed to daily salinity increases of 1 to 2 ppt through the addition of NaCl.

Relative to samples receiving no salt supplement, densities of *Microcystis* spp. declined in salinity-stressed samples. Chlorophyll concentrations declined slightly relative to assemblages receiving no salt additions while carbon fixation was reduced in salinity-stressed assemblages. These results suggest that salinities from 0.5 to 7 ppt could limit the distribution of *Microcystis*-dominated blooms.

Experiments were done on *Oscillatoria acuminata* to determine the effect of different salinity levels on growth. Growth was measured in terms of chlorophyll 'a' at different salinity levels. Maximum growth was observed at higher salinity (35 ppt) whereas, minimum growth was observed at lower salinity (5 ppt).

These results were in agreement with findings of Kumar-Rai and Abraham (1993). Thus, in general, increased salinity reduces the amount of cyanobacteria growth but each species has its own optimum sodium chloride concentration.

CONCLUSION

Subjecting the cyanobacteria isolates to different physicochemical conditions altered their growth rates. The cyanobacteria species have optimum growth at room temperature (23 ± 2°C) and could also grow at 50°C and hence, can be described as thermotolerant. The species have optimum growth at pH 6 up to pH 10. They may therefore be considered to be alkalitolerant whose growth is optimum at pH <8.5 and maximum pH >9.0.

The species inhabit hypersaline waters (30% w/v) of the lake and yet appeared to have optimum growth rate in lower salinity (0.0 to 0.3% w/v) conditions of culture media. Many of them have optimal growth at NaCl concentration of less than 0.2 mol/L (0.12% w/v) hence, could be classified as halotolerant.

Cyanobacteria therefore, may not be distinctly classified as marine or fresh water but can be described in terms of their ability to tolerate certain conditions, such as the thermotolerant *Synechococcus* sp, the alkalitolerant *Microcystis* sp and the halotolerant *Arthrospira* sp. This may explain why cyanobacteria are known to inhabit almost every type of environment.

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