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Effect of Aluminum and pH on the Growth of *Anacystis nidulans*

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Anacystis nidulans is a typically rod-shaped unicellular cyanobacterium. It is an obligate photoautotroph whose photosynthetic apparatus is very similar to eukaryotic chloroplasts in functional and molecular aspects (Fogg 1973).

When industrial,agricultural and municipal wastes enter a body of water, the populations of algae and other related organisms in the water increase. The presence of cyanobacteria in fresh water, such as the dense blooms that grow in polluted lakes, pose a threat to the lake's ecosystem. The algal populations of a river, lake or stream can provide an indicator of the degree of pollution and indirectly of the effect of different conditions in nature, such as acid rain or toxic metals. Monitoring the level of cyanobacteria populations in the water has been suggested as an indicator for water pollution. (Raizada 1989)

Of the toxic materials discharged, trace and heavy metals are of major concern. Metals are introduced into the environment by industrial agricultural and municipal wastes as well as by natural runoff. Metals emitted into the environment from combustion can also find their way into waterways.

Aluminum is one of the most abundant metals in the earth's crust. It is reported that water draining from rock strata associated with coal seams may contain acid salts of aluminum, iron and manganese (Casarett 1980) The toxic effects of aluminum on plant growth have long been known. Aluminum is a major growth limiting factor in acid soils containing significantly lower concentrations of exchangeable hydrogen and aluminum ions (Cribben 1977).

Several concentrations of aluminum, as well as various

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pH values were studied, to determine the extent to which some pollutants will effect the growth of *Anacystis nidulans*. Since reports have indicated that EDTA influences the effect of many metals, studies were performed with and without EDTA(Fry et al 1985;).

MATERIALS AND METHODS

A. nidulans 625 cultures, obtained from Dr. Roy McGowan of Brooklyn College, New York, and maintained as stock cultures in 5 ml aliquots, were inoculated into sterile flasks containing 100 ml of Mauro's Modified Medium (3M)(Kratz and Myers 1955). Various concentrations of aluminum sulfate $Al(SO_4)_3 \cdot 5H_2O$ as a stock solution and diluted to achieve final concentrations of 1,5,20 and 50 ppm, were added to the flasks. Cultures were grown at ambient temperature under fluorescent light with continuous slow agitation until stationary phase was achieved. Growth of the cultures was determined by two methods: 1. direct count using a Spencer hemocytometer or 2. indirect turbidometric reading using a Beckmann spectrophotometer at 750 nm. Cultures were checked for contamination by plating on nutrient agar. PH readings were taken at the beginning and end of each experiment. Another set of experiments was carried out with the same protocol as described above and 1,5,20,50 and 100 ppm aluminum. In this set, the pH value of the cultures was adjusted to pH 7.9 before autoclaving using KOH or NaOH.

An additional set of experiments was performed to determine the effect of pH on the growth of *A. nidulans*. The pH of flasks containing 3M was adjusted with either 1N NaOH or 1N HCl to achieve the following pH values:2.4,4.5,6.5,7.9,10.0,11.0,11.5 and 12.0. PH 7.9 is considered to be the optimum for *A. nidulans* cultures(Kratz and Myers 1955). All cultures were grown and monitored as described above. Viability testing of each culture was also carried out, using streak method on 3M agar. The cell morphology of each culture was observed at 1000X magnification.

RESULTS AND DISCUSSION

The effect of different pH values:2.4, 4.5, 6.5, 7.9, 10.0, 11.0, 11.5, 12.0 was studied by monitoring changes in cell density and turbidity for 5 days (Fig 1). No cell growth can be detected at pH 2.4 and 12.0. At 4.5 and 11.5 growth was severely reduced. The optimum range is 6.5 to 7.9 (optimum at 7.9).

The pH effect on cell morphology and viability was studied and the pH of the cultures was measured at D1

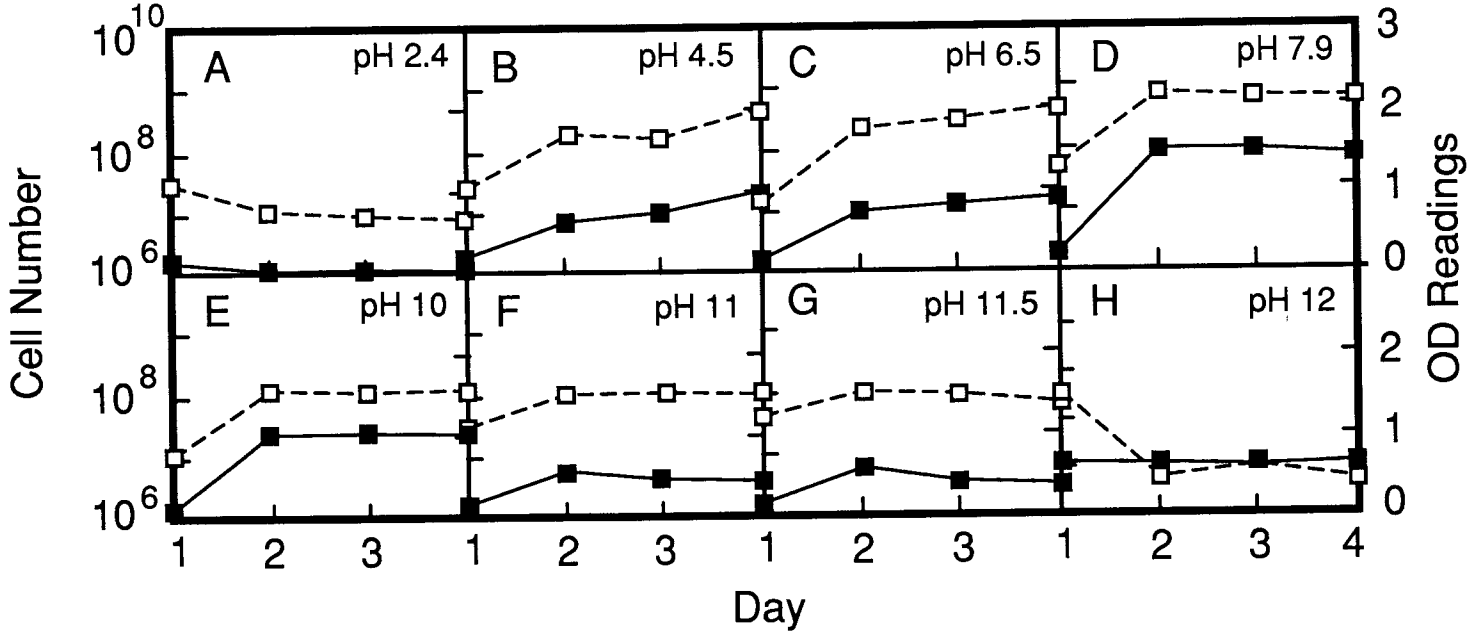

Figure 1. Effect of different pH values on the growth of *A. nidulans* a)pH2.4 b)4.5 c)6.5 d)7.9 e)10 f)11 g)11.5 h)12 □——□ O.D.750 ■——■ Cell Number

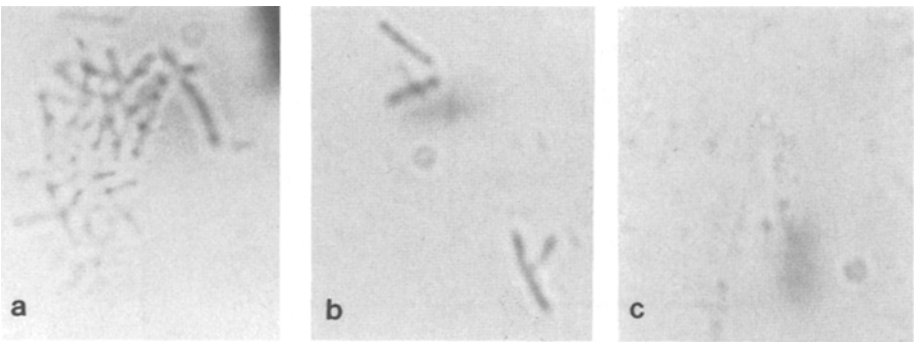

Figure 2. Effect of pH on Cell Morphology
a) pH 4.5 b) pH 7.9 c) pH 11.5

Table 1. Examination of the morphology and viability of *A. nidulans* growth under various pH conditions.

pH		Morphology	Viability	
Day 1	Day 6	Day 3	Day 6	
2.4	2.4	tiny*	thick wall	-
4.5	8.0	small*	slender	+
6.5	8.0	small*	elongated	++
7.9	8.1	moderate	elongated	+++
11.5	9.0	elongated filaments	swollen,long filaments	-
12.0	12.0	small	cell debris	-

*aggregation of small cells

and D6. The results are summarized in Table 1 and seen in Fig.2. The final pH at D6 showed that pH values can be altered by the cyanobacter, from the extremes of 11.5 or 4.5, to the more favorable range (7.5-8.5). No adjustment occurred at 2.4 or 12.0. These results suggest that algal growth will be suppressed in the presence of acid rain (pH 3.0-4.5). At highly alkaline conditions (11.5), cell size increased by 2 to 3 times, rather than cell numbers increasing.

Since the cultures could adjust the pH from the extremes back to the optimum range, some regulatory mechanism, such as a buffer system, must be present. While the extreme conditions can cause alterations in the morphology of the cells, these findings suggest that when environmental pH varies, *A. nidulans* may be able to undergo alterations in its metabolic pathways to produce products which will adjust the environmental pH and therefore allow the organism to grow over a wide range of pH values.

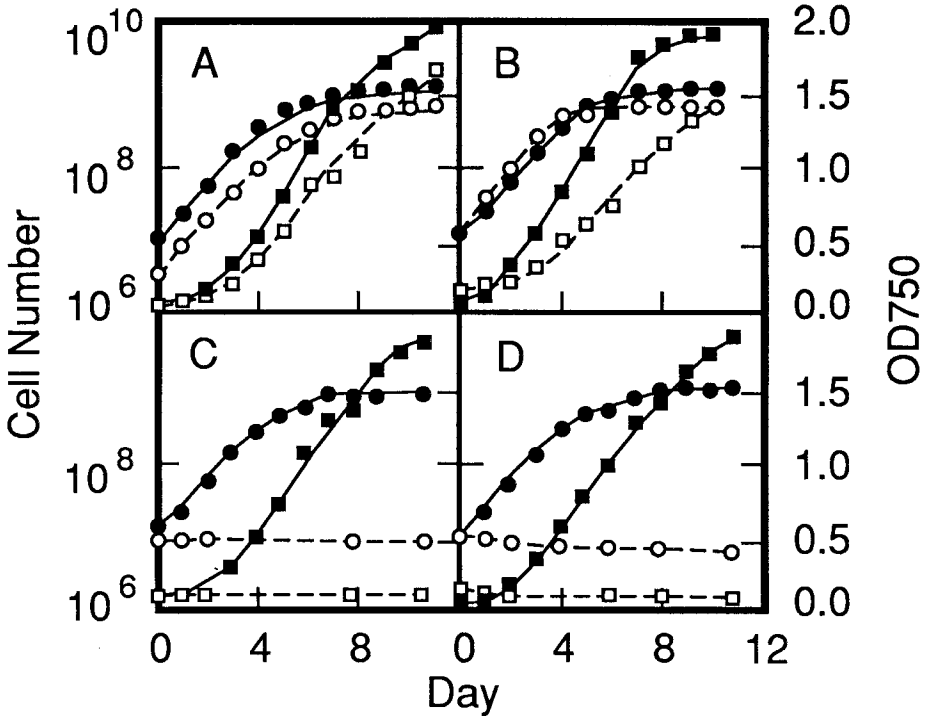

Figure 3. Effect of Aluminum without pH adjustment on growth of *A.nidulans* a) 1 b)5 c)20 d)50 ppm

The results of the addition of 1, 5, 20 and 50 ppm of aluminum can be seen in Fig 3. Aluminum caused a shock effect in the early stage of growth at all concentrations. At 1 and 5 ppm, there was a delay in the onset of exponential phase of growth. However, concentrations of 20 and 50 ppm completely inhibited the growth of *A. nidulans*. The pH values of the media containing 1,5,20 and 50 ppm aluminum were 7.3, 6.65, 3.6 and 3.35 respectively.

To determine if the lowered pH values at the higher aluminum concentrations might have caused the lethal results, further experiments were done using 1,5,20,50 and 100 ppm aluminum. In these experiments KOH was added to adjust the pH to 7.9(Fig.4). These results indicate that different concentrations of aluminum did affect the growth of *A. nidulans*, regardless of pH adjustment. For concentrations of 1 and 5 ppm, the results were very similar to Fig.3A and 3B. For concentrations of 20 and 50 ppm the results were very

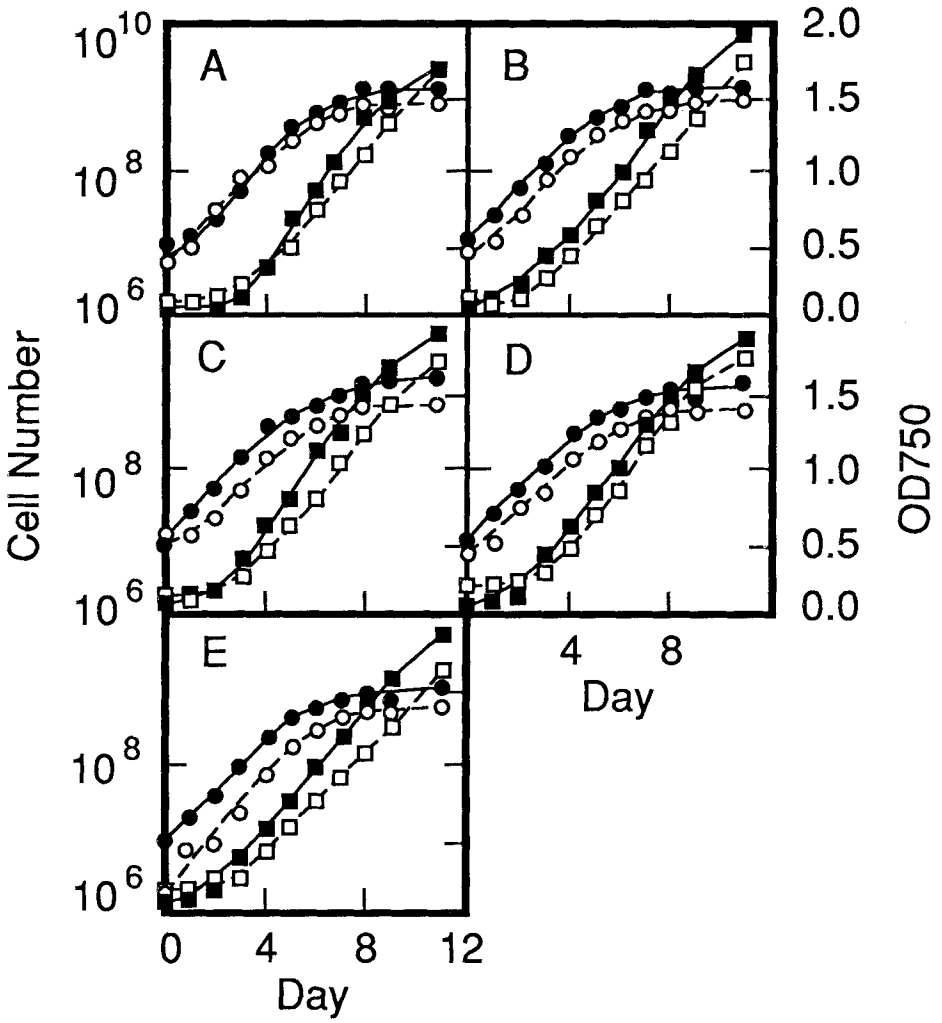

Figure 4. Effect of Aluminum with pH adjustment on growth of *A.nidulans* a)1 b) 5 c) 20 d) 50 e) 100 ppm

different from Fig. 3C and 3D. Cultures were even able to grow in 100 ppm. The effect of aluminum was much less in the cultures with pH adjustment that in cultures without adjustment. This suggests that the higher concentrations of aluminum may be due in part to the acidic conditions, or that the aluminum ionic form may be more toxic in the acidic condition.

It has been reported that EDTA is a good metal chelating agent (Casarett 1980). Media containing 50

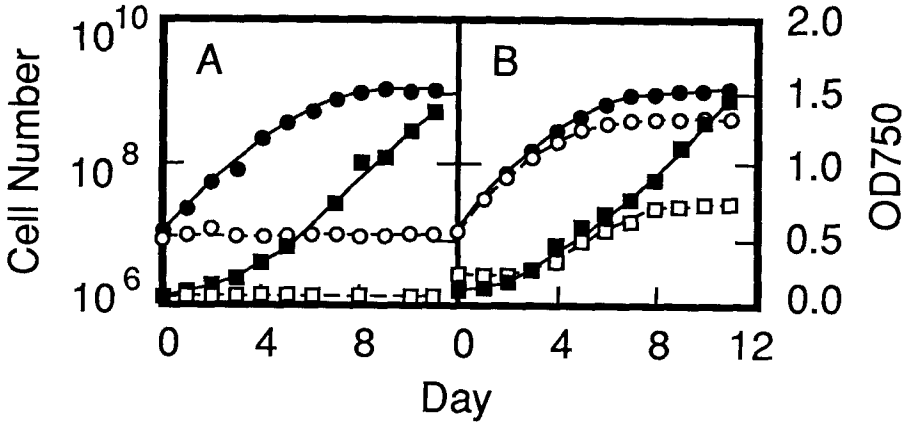

Figure 5. Effect of Aluminum without EDTA with and without pH adjustment (50 ppm Al)
a) with pH adjustment b) without pH adjustment
□——□——□ turbidity of treated culture
○——○——○ cell number of treated culture
■——■——■ turbidity of control
●——●——● cell number of control

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ppm Al were prepared without EDTA and with and without pH adjustment (Fig 5). These results indicate that in both conditions, growth was very similar to Fig 3D without pH adjustment and Fig 4D with pH adjustment. This indicates that EDTA did not change the effect of aluminum on the growth of *A. nidulans*.

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