MOVEMENT IN THE CYANOPHYCEAE

THE EFFECT OF pH UPON MOVEMENT IN OSCILLATORIA

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As part of a comprehensive study of the nature and mechanism of the movements of Cyanophyceae, the effect of hydrogen ion concentration upon the velocity of translatory movement in Oscillatoria formosa Bory was determined under controlled conditions. The profound influence of the hydrogen ion upon ameboid movement has been demonstrated (Pantin, 1923), and the effect of pH upon various metabolic processes of motile autotrophic organisms has been shown by a number of investigators. Among others, Maertens (1914) has reported that Oscillatoria grows best at slightly alkaline reactions in suitable culture media. The lethal effect of acids upon Oscillatoria was noted by Schmid (1923), and a negative chemotactic response was observed by Fechner (1915). However, no quantitative information concerning the speed of movement of this alga in relation to hydrogen ion concentration has been found in the literature.

Methods

Oscillatoria was collected from the mud flats along the Charles River (Cambridge, Mass.) and grown on 1 per cent nutrient agar impregnated with inorganic salts according to a formula employed by Uspensky and Uspenskaja (1925). The stock cultures were isolated from a single filament and grown in Petri dishes under diffuse daylight.

For experimental purposes, the following procedure was adopted: the dishes were wrapped in clear cellophane to conserve the moisture content of the agar and placed in a constant temperature incubator (about 18°C.) some 30 cm. distant from a 25 watt frosted bulb. After a minimum period of 15 hours' adaptation to these conditions, the alga was deemed ready for transfer to the observation cells. The cells employed were of the Van Tienhoven type, each consisting of a glass ring

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cemented with Picein to a microscopic slide and a round glass cover-lid. After carefully greasing the upper rims with vaseline, the cells were filled with the stock culture solution. Small bits of agar containing the Oscillatoria filaments were transferred with a platinum needle to a series of the cover-lids wet with drops of the same culture solution. Then the inoculated covers were dropped carefully upon their respective cells and sealed without the formation of air bubbles. The series of preparations was then placed in an inverted position in the incubator for a period of about 5 hours, until the algal filaments had begun to glide freely over the inner surface of the cover-glass. Different experimental solutions could be substituted in the cells by merely sliding the covers aside sufficiently to permit entrance of a fine pipette. The filaments adhering to the glass formed a suitable preparation for microscopical examination.

The source of illumination for the microscopical observations consisted of a 100 watt tungsten ribbon filament bulb focused with a condensing lens onto the plane substage mirror. From the mirror the light was reflected upward to a finely ground glass plate placed just beneath the stage, and thence directly illuminated the cell containing the organisms. An intensity of 26 foot candles was used (measured with a Weston photronic cell placed on the microscope stage). Infra-red radiation was effectively removed (Coblentz, 1911) by interposing between the light source and the microscope a glass cooling cell containing a 2.2 cm. thickness of 2.5 per cent solution of copper chloride in water.

All observations were made at 22°C. with the microscope placed in a constant temperature box which could be kept within a variation of ± 0.1°C. The time required for a filament to traverse one space (16.6 microns) of the ocular micrometer was measured with a stop-watch. From the average of two readings for a filament, the velocity was computed in terms of microns per second. Ten different filaments, which appeared free to move in an unimpeded manner, were chosen for each given set of readings. A set of observations could usually be made in 10 to 15 minutes, depending upon the speed of the Oscillatoria as well as upon the dexterity of the observer. The fact that repeated observations at intervals could not be performed upon the same filaments is not considered a serious objection, since similar methods of observation upon Oscillatoria have hitherto given clear results (cf. Crozier and Federighi, 1924).

The solutions were always made up in large volume with glass-distilled water; aliquot parts were adjusted to the desired pH with acid or base. The lower pH values were determined electrometrically with the quinhydrone electrode and in the solutions above pH 7.3 with the hydrogen electrode.1

A series of solutions ranging from pH 4.2 to 11.2 was obtained by the addition of N/10 HCl or N/10 NaOH to 100 cc. portions of Us- pensky’s solution modified to include K2HPO4. The pH of this stock

1 Thanks are due Dr. P. S. Tang for his kind assistance with the hydrogen electrode determinations.
solution, as made up, was 7.05; the concentration of salts was 0.211 gm. per liter. From two to six separate cells were set up for each pH value at different times and a set of readings was made on ten filaments in each cell, starting 5 minutes after initial immersion of the organisms in the test solutions. At the end of an hour, during which time the cell remained in the light on the microscope stage, another set of readings was made. Thus a total of 1600 readings were made upon forty different cells in the course of this experiment.

The means of the observations at the beginning and end of the hour showed close agreement for each pH value, with indications of progressive inhibition in the extreme pH ranges. In Fig. 1 there are plotted the mean rates of all readings taken at each pH after approx-
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imatively 1 hour. Fair activity is indicated between pH 6.0 and 9.8, but beyond these points the decrease in velocity is striking. A wide favorable range appears to lie on the basic side of neutrality.

Since this solution contained carbonates and relatively high ratios of Ca and Mg which precipitated out at the higher alkaline reactions, a similar experiment was performed using a different type of formula in making up the stock solution. This experiment was planned to test the influence of the time factor upon movement in solutions covering a wide pH range. A modification of the solution employed by Maertens (1914) in his investigation of the optimum H+ ion concentration for the growth of blue-green algae was utilized as a stock. The composition of the solution was as follows: Pyrex-distilled water—1000 cc.; KNO₃—0.1 gm.; MgSO₄—0.65 gm.; K₂HPO₄—0.2 gm.;

![Graph showing mean rates of translatory movement of Oscillatoria after immersion for 2 hours (open circles) and for 8 to 10 hours (solid circles) in solutions of different pH. Each point represents the mean value for ten filaments.](image-url)
Ca(NO₃)₂—0.04 gm.; and Fe₂(SO₄)₃—0.0007 gm. (total salt content of 0.3907 gm. per liter). The pH of this solution, as made up, was 7.3; from it a series of thirteen different solutions was adjusted with N/10 HCl or N/10 KOH to cover the range pH 4.35 to 11.2. No precipitate could be seen in the alkaline solutions and the maximum amount of acid or base required to obtain the extreme ranges was considered negligible from the viewpoint of osmotic effects of the total salt concentration.

Movement of the Oscillatoria was observed at repeated intervals after immersion in the respective pH solutions; i.e., at approximately 5 to 15 minutes, 2 hours, 8 to 10 hours, and 24 to 26 hours. During the intervals between readings the cells were kept in the incubator and at from 10 to 15 minutes preceding each of the repeated sets of observations fresh solutions were placed in the cells. Several sets of
data were procured from different cultures, and though the pH range showed close agreement in all, the actual rates varied from one culture to another, probably due to varying nutrient relations or different water content of the agar. The results of one series of observations for a single culture are presented in Figs. 2 and 3.

The data show favorable conditions for movement in a relatively wide alkaline range up to about pH 9.5. Below neutrality, activity appears to be checked in the region of pH 6.4. Furthermore, at unfavorable pH values the inhibition is progressive with exposure time. That this inhibition is due to pH, and not to the method of handling nor to improper adjustment of the dissolved gases, is supported by the continuous high rates for 24 hours in the favorable alkaline range mentioned.

It was noted that in acid solutions where inhibition was complete there occurred a shrinkage of the protoplasts followed by dissolution of the filaments. In the inhibiting alkaline solutions there was on the contrary a suggestion of swelling in the vicinity of the transverse walls, in such a way as to cause separation of the apical cells by a wide hyaline area.

It appears that movement occurs within the pH range which has been indicated as best for the growth of these organisms. The actual velocity of locomotion as well as the pH range agrees well with the data reported for ameboïd progression in contact with glass. Of course the effect of the hydrogen ion would be modified, were other factors altered; i.e., the salt content, temperature, light, kind of substratum, etc. The effect of pH in relation to other variables influencing motility demands further investigation.

**SUMMARY**

The effect of pH upon the velocity of translatory movement of Oscillatoria formosa Bory in inorganic culture solutions was determined. Unhindered movement occurred in the range of about pH 6.4 to 9.5. Above and below these limits inhibition was marked.

In the unfavorable acid and alkaline ranges inhibition was progressive with exposure time; in the favorable range continuous movement was maintained for 24 hours.
CITATIONS