APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1985, p. 73–78
0099-2240/85/010073-06$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 49, No. 1

Factors Affecting the Germination of Akinetes of *Nodularia spumigena* (*Cyanobacteriaceae*)

ANN L. HUBER
Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Western Australia 6009

Received 7 March 1984/Accepted 6 August 1984

Nutritional and physical factors which influence the germination of akinetes of *Nodularia spumigena* (Cyanobacteriaceae) were examined. Low concentrations of phosphorus (<0.9 μM) were required for germination. Nitrate had no effect, but ammonia, at concentrations of >45 μM, inhibited germination. Salinities of >20‰ were inhibitory to germination. Optimum temperatures were 22°C or greater. Germination did not take place in the dark, but only very low light intensities (0.5 microeinstein m^{-2} s^{-1}) were necessary to initiate germination. Red light (620 to 665 nm) was required. More than 24 h of continuous exposure to light was necessary for any significant germination to occur. The conditions for germination corresponded with conditions in the Peel-Harvey Estuary, Western Australia, 2 to 3 weeks before large summer *Nodularia* blooms.

Akinetes of cyanobacteria are differentiated resting-stage cells which generally contain high concentrations of cyanophycin granules, polyhedral bodies, and glycogen (cf. 14, 23), lack polyphosphate granules (16) and gas vesicles (14), and are surrounded by a thick, multilayered envelope (cf. 7, 12). The nucleic acid content of akinetes relative to vegetative cells is apparently very different for different species (see reference 13). Grilli Caiola and de Vecchi (5) proposed two types of akinetes for *Nostoc* strains isolated from coralloid roots of cycads. The first represented vegetative cells which had temporarily stopped growing due to adverse conditions but were able to germinate immediately, whereas the second represented mature akinetes able to withstand unfavorable conditions for extended periods of time.

The mechanisms of germination have been examined in some detail by Braune (3, 4), Grilli Caiola and de Vecchi (5), Miller and Lang (12), and Wildman et al. (26). However, the factors involved in initiation of germination are not as well understood. Light has most commonly been implicated (1–3, 8, 15, 16, 21, 23, 27). Temperature and nutrients have also been shown to be triggering factors (15, 16, 25–27).

The role of cyanobacterial akinetes as perennating structures has been suggested by Adams and Carr (1), Roelofs and Oglesby (21), and Reynolds (18, but see reference 19). However, this point of view has been opposed by Fogg (1969, cited in reference 15) and Pandey and Talpasayi (15). As pointed out by Nichols and Adams (13), the significance of akinetes "in preserving the cyanobacterial stock during periods of non-vegetative growth is probably a function of the species and the particular environmental conditions." In the Peel-Harvey Estuary, Western Australia, it is likely that akinetes are significant in providing inocula for the extensive summer blooms of *Nodularia spumigena* which occur there (6). Sediment akinetes and their relationships to cyanobacterial blooms have also been discussed by Kappers (8), Livingstone and Jaworski (10), Reynolds and Walsby (20), Rother and Fay (22), and Wildman et al. (26).

In the 7 years since a study of the Peel-Harvey estuarine system has begun, *Nodularia* blooms have occurred in the estuary after the following events. Diatom blooms, which have taken up phosphorus supplied by winter runoffs, senesce and decompose on the sediment surface, thus releasing nutrients. At the same time the water clears and temperatures and salinity increase. One or a combination of these events appear to trigger akinete germination, which results in a massive bloom 2 to 3 weeks later. It is important to determine what factors are significant in the germination of cyanobacterial akinetes to understand more fully their role as inocula for blooms. Because many of these parameters undergo simultaneous changes in situ, it is necessary, in the first instance, to examine their effects separately.

The aim of the present study was to examine the effects of nutritional and chemical factors (phosphorus, nitrogen, iron, and salinity) and physical factors (temperature, light intensity, wavelength, and exposure time) on the germination of *N. spumigena* akinetes.

MATERIALS AND METHODS

Akinete cultures. *N. spumigena* Mertens was isolated from the Peel-Harvey Estuary and grown in modified BG11-2 medium or estuary water-based BG11-2 (Table 1). Cultures were allowed to remain in stationary and declining phases for 1 to 2 months to allow a large proportion of akinetes to form. Cultures were then placed in the dark at room temperature and allowed to decompose for approximately 1 month. Vegetative filaments tended to remain attached to the akinetes, and other methods of separation did not yield high concentrations of free akinetes. When the vegetative cells were sufficiently decomposed, the cultures were filtered through 1.2-μm membrane filters (Millipore Corp.). Most of the akinetes were retained on the filters while the cellular detritus passed through. After several washings with deionized water, the akinetes were resuspended in a small volume of deionized water. Suspensions could be stored at 4°C in the dark for several months without losing viability.

Germination testing. Akinete suspension (0.1 ml) was added to 2 ml of BG11-2 medium in 15-ml screw-capped Pyrex culture tubes. Phosphorus, nitrogen, and iron concentrations used were those of the BG11-2 medium unless otherwise stated. The salinity was 5‰ except in the salinity trials. Normal light and temperature conditions were 25 microeinsteins m^{-2} s^{-1} and 21°C.

After 5 and 8 days of incubation, the percentage of akinetes was determined by counting the number of germinated akinetes and hormogonia in a total of 100 to 400 akinetes. Standard deviations for individual counts within one tube have been plotted. The variability in germination

TABLE 1. BG11-2 medium[a]

Component	Final concn (g liter^{-1})
NaCl	5.0
K_2HPO_4	0.039
$MgSO_4 \cdot 7H_2O$	0.075
$MgCl_2 \cdot 6H_2O$	0.20
$CaCl_2 \cdot 2H_2O$	0.0268
$NaHCO_3$	0.02
$FeCl_2$ sol[b]	1 ml
Micronutrient mixture[c]	1 ml
TAPS buffer (Sigma Chemical Co.)	0.05 M[d]

[a] Modified from Hughes et al. (a).
[b] $FeCl_2$ solution: 0.365g $FeCl_2$ and 0.6 g of Na EDTA in 100 ml of distilled water.
[c] Micronutrient mixture (milligrams 100 ml^{-1}): $CoCl_2 \cdot 6H_2O$, 0.08; $MnCl_2 \cdot 4H_2O$, 0.5768; $NaMoO_4 \cdot 2H_2O$, 0.1261; H_3BO_3, 0.05725.
[d] pH adjusted to 8.6 with NaOH.

among independent controls (untreated akinete suspensions) was measured. The percent germination and standard error were 84.4 ± 2.1% (n = 5), indicating good reproducibility within a trial. In some cases, the mean length of the hormogonia was also determined. All experiments were repeated to confirm the results, except for the light exposure time and nitrate-nitrogen trials. In these cases, replicate tubes were examined. Standard errors between replicate tubes have been plotted.

Treatments. Phosphorus (K_2HPO_4) additions ranged from 0 to 224 μM. The effects of both ammonia-nitrogen (NH_4Cl, 0 to 143 μM) and nitrate-nitrogen ($NaNO_3$, 0 to 714 μM) additions were examined. Iron concentrations (in 16 μM EDTA) ranged from 0 to 65 μM.

Salinity was adjusted with NaCl; the range tested was from 0 to 40‰. For the temperature trials, the akinete suspensions were placed on the surface of an aluminum temperature block. The temperature range tested was from 12 to 24°C.

Light intensities tested ranged from 0 to 99 microeinsteins m^{-2} s^{-1}; cool white fluorescent tubes were used as the light source. A Lamda Instruments LI-185 photometer, having a quantum sensor, was used for the light intensity measurements. To determine the effect of selected wavelengths, the culture tubes were wrapped with yellow, orange, red, purple, blue, or green cellophane. The spectral properties of each of the colors were determined by absorbance and percent transmittance scans from 850 to 315 nm on a Pye Unicam SP8-150 UV/VIS spectrophotometer. Tubes were placed at the distance required to give a light intensity of 8 microeinsteins m^{-2} s^{-1}. The light exposure time required for initiation of germination was determined by incubating the akinetes in light (25 microeinsteins m^{-2} s^{-1}) for 10 min to 48 h. After the required exposure, the tubes were placed in the dark. After the longest exposure time (48 h), all tubes were incubated for a further 5 days before determining the percent germination.

RESULTS

Phosphorus. To germinate, *Nodularia* akinetes required very small concentrations of phosphorus (Fig. 1). In trial 2, no germination occurred in the absence of added phosphorus and maximum germination took place in the presence of 0.9 μM phosphorus. In trial 1, 68% germination occurred without added phosphorus. However, it is possible that not all traces of phosphorus were removed during the preparation of the akinete suspensions.

Nitrogen. Both ammonia and nitrate nitrogen were examined for their effects on germination. The results are presented in Fig. 2a to c. For ammonia, in both trials, akinete germination was inhibited by concentrations of >45 μM. At 71 μM, there were 36 and 0% germinations in trials 1 and 2, respectively (Fig. 2a). It was also noted that the hormogonia length was dependent on ammonia nitrogen concentration (Fig. 2b). In trial 2, the maximum mean length occurred at 4.5 μM, and the minimum occurred without added nitrogen. The mean hormogonia length was shorter after 4.5 μM, but then remained constant until germination no longer took place.

In contrast to ammonia, nitrate had no effect on akinete germination (Fig. 2c). The maximum initial concentration tested was 714 μM, approximately five times the maximum tested for ammonia.

Iron. In both trials, iron stimulated akinete germination by approximately 30% over the range of concentrations tested. However, 50 to 55% germination did occur in the absence of added iron (Fig. 2d). The difference between germination in the presence or absence of added iron was significant at the 0.95 level (Student's t test).

Salinity. High salinities (above 20‰) greatly decreased akinete germination (Fig. 3b). At 40‰, no germination occurred. It was also noted that the mean hormogonial length was shorter at 30‰ than at lower salinities (data not shown). This is likely to have been a function of both slower germination and reduced growth rates.

Temperature. Germination rates increased with temperature (Fig. 3a). At 5 days at 12°C, no germination had taken place in either of the trials. The apparent discrepancy between trials 1 and 2 in Fig. 3 is the result of a very sharp increase in germination rate at between 15 and 16°C. By day 5, 2 and 60% germination had occurred at 15°C in trial 2 and at 16°C in trial 1, respectively. The effect of low temperatures was to slow down the germination rate rather than to completely inhibit it. By 8 days in trial 1 at 12°C, 40% of the akinetes had germinated compared to 0% 3 days earlier.

Light. Three aspects of light were examined with respect to their effects on akinete germination: light intensity, wavelength, and exposure time.

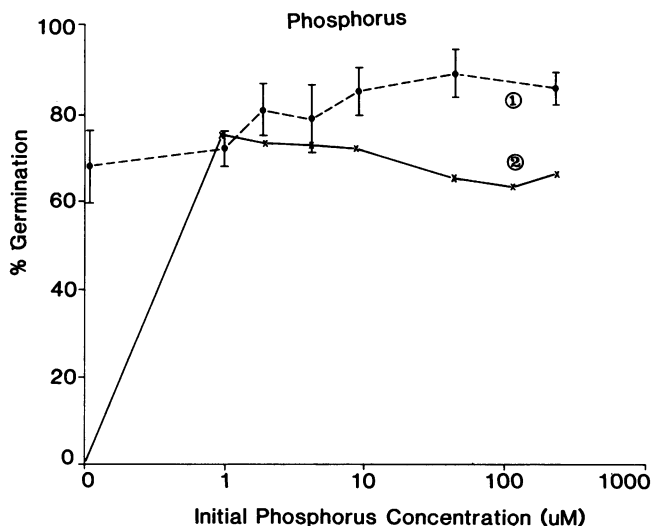

FIG. 1. Effect of initial phosphorus concentration on *Nodularia* akinete germination, trials 1 (O) and 2 (O). Standard deviations are shown for trial 1.

Akinetes failed to germinate in the dark. However, as little as 0.5 microeinstein m^{-2} s^{-1} allowed significant germination (29% in trial 1 and 56% in trial 2 at 5 days) (Fig. 3c). Trial 1 reached maximum germination at 9 microeinsteins m^{-2} s^{-1}, but the same intensity in trial 2 only resulted in 51% germination. It was also noted that the hormogonial length increased with light intensity, being maximum at 80 microeinsteins m^{-2} s^{-1} in trial 1 at 5 days. This is likely to be a function of both early germination and rapid growth rate. By day 8 in trial 2, however, the high light intensity had caused the cells to become bleached and unhealthy.

The minimum time of exposure to light which was required for germination was 24 h at 9 microeinsteins m^{-2} s^{-1} (Fig. 3d).

The germination rate was not affected by any of the color filters except green (Table 2). With the other filters, there appeared to be a slight inhibition of germination after 5 days, but by 8 days (trial 1) germination had reached its maximum. Germination in the presence of the green filter, however, was only 50 to 60% at both 5 and 8 days. The peak wavelengths absorbed by each of the filters are listed in Table 2. The green filter absorbed both red (620 to 665 nm) and blue (405 nm) wavelengths. Neither the blue nor the yellow filters caused any significant inhibition of germination. However, a scan of the percent transmittance of the filters indicated that the green filter absorbed 97% of the light at the red wavelengths and 100% of the light at the blue wavelength. The blue filter absorbed only 76% of the red light, and the yellow filter absorbed 99% of the blue light. It appears, therefore, that it is the red wavelengths which are critical for germination of *Nodularia* akinetes.

Field data. The dates when the bottom water conditions were appropriate for rapid akinete germination from 1978 to 1983 are presented in Fig. 3. Bottom water conditions, taken from a report of Lukatelich and McComb (11), have been used to determine these dates because this was the only continuous data set available that approximated the environment surrounding the akinetes. The following criteria for rapid akinete germination have been used: temperatures of >16°C; salinities of <20‰; 0.3 μM PO_4 P or more; and <40 μM NH_4 N. Iron data were not available and light data were insufficient. The presence or absence of nitrate has been assumed to be unimportant because of the laboratory studies. The pH of the bottom waters has always been 7 to 9, and oxygen has always been present, though at times at low concentrations.

Nodularia blooms were usually reported 2 or 3 weeks after "suitable" conditions existed in the estuary (Table 3).

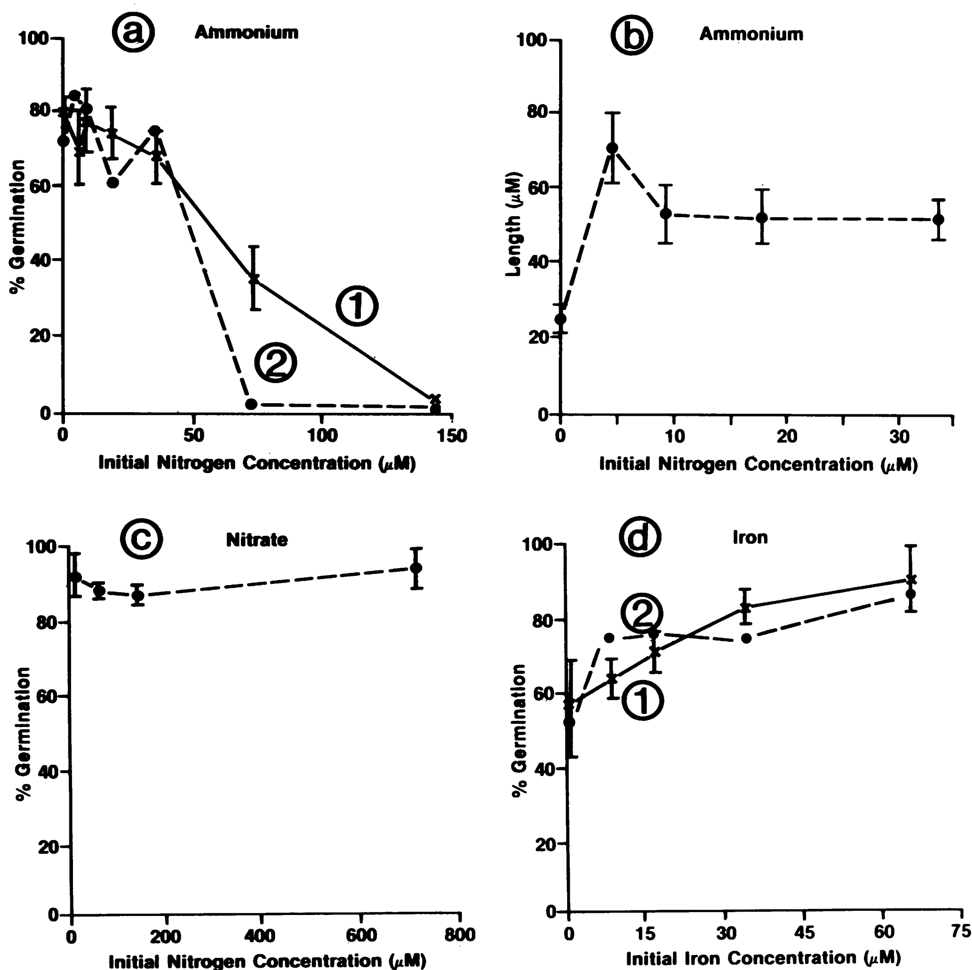

FIG. 2. Effects of ammonia nitrogen on percent germination (a) and hormogonial length (b) and of nitrate nitrogen (c) and iron (d) on percent germination of *Nodularia* akinetes. Standard deviations are given for trial 1 for ammonia and iron. Standard errors are given for nitrate.

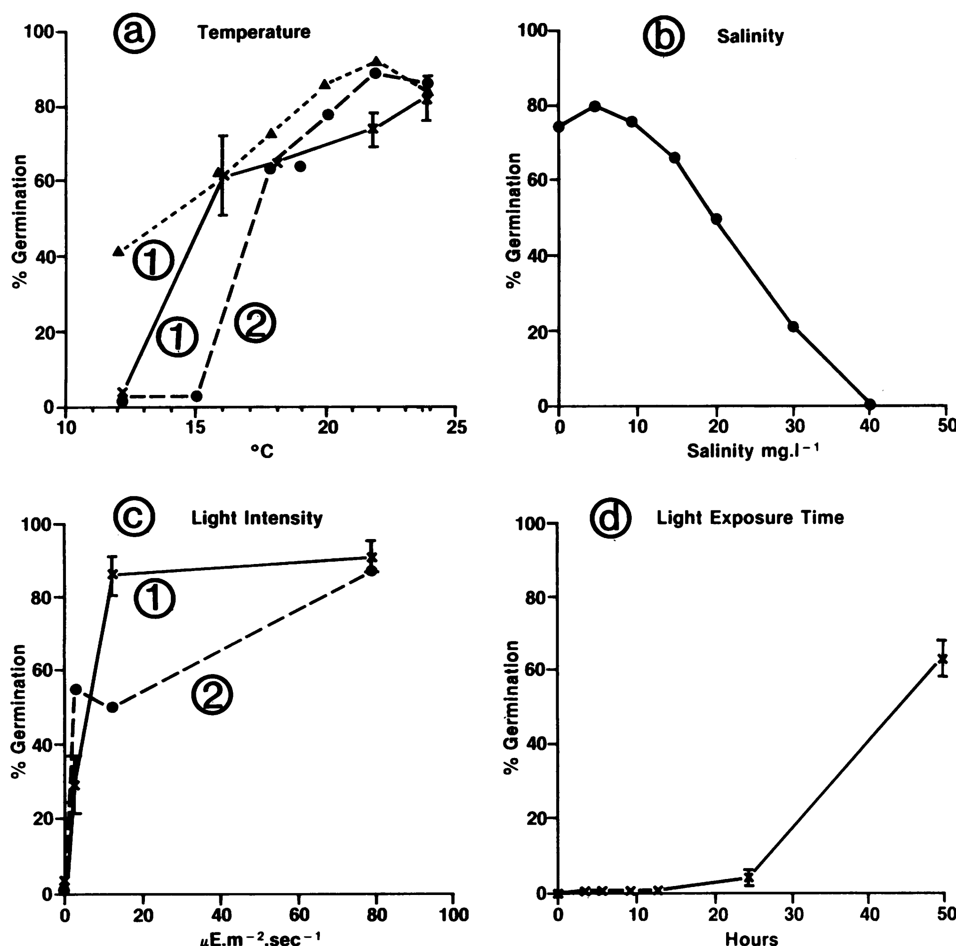

FIG. 3. Effect of temperature on *Nodularia* akinete germination at 5 (trials 1 [×] and 2 [●], respectively) and 8 (trial 1) days (a). Standard deviations for counts are shown. (b–d) Effects of salinity (b), light intensity (c), and light exposure time (d) on *Nodularia* akinete germination. Standard deviations for counts are shown. μE, Microeinsteins.

In 1979 there was only 1 day at one site when all recorded conditions were appropriate for germination, and no bloom occurred in that summer. During most of September and October 1979, phosphorus concentrations were always <10 μg of P liter^{-1}. All other parameters were within the range suitable for germination. In the spring, when a bloom did not occur several weeks after the first suitable date(s), e.g., 1981, the intervening period until the next appropriate date was unsuitable for germination or growth, frequently because of cold temperatures. Phosphorus was the parameter most often outside the germination range in the years and at locations where blooms did not occur, but not always. For example, in January 1980, phosphorus concentrations exceeded 10 μg liter^{-1} in some locations. However, salinities were too high. During winter and early spring, temperature was often limiting. It is also interesting to note that, in February 1982, due to unseasonal heavy rainfall, conditions again became appropriate for germination. The parameter most significantly changed with respect to the germination criteria being applied was salinity. At this time the *Nodularia* bloom, which had been declining, began to regrow. Part of this regrowth may have been due to the germination of that season's akinete population. The lag in regrowth was only 1 week in that case.

DISCUSSION

Apart from the work of Rai and Pandey (16) and Yamamoto (27), no systematic examinations of the effect of any nutrients on germination appear to have been done. Miller and Lang (12) obtained germination of *Cylindrospermum* akinetes by placing sporulated cultures into fresh medium. Thus, by implication, nutrients would seem to be important

TABLE 2. Akinete germination response to light wavelength

Filter color	Peak absorbance (nm)	% Transmittance at maximum absorbance	% Germination		
			Trial 1		Trial 2 (5 days)
			5 days	8 days	
No filter			89	86	89
Yellow	438	1	84	81	81
Orange	420, 544	2, 27	76	88	72
Red	522, 408	0, 1	78	89	73
Purple	559–589	21	81	88	77
Blue	600–632	24	74	81	75
Green	620–650, 405	3, 0	51	50	57

TABLE 3. Dates[a] when bottom water conditions were appropriate for akinete germination, 1978–1983[b]

Yr	Station					
	31	1	58	2	7	4
1978	ND[c]	O10, 24	ND	O10		O10, 24
1979	S25					
1980	O10–N1	S23 O22				
1981	A25 S15–O27 F2–9/82	A25 S22 O13–27 F2, 9/82	A25 S22 F9/82	S22–O13	A25	A25 S15
1982	A31 S21–12 N16–23	A31 S21–O5 O26–N2	A17, 31 S28 O12	S21–28	S21–28	A31 S28
1983	S13–27 O4–11	S13–27 O4	S13–27 O4–11	S20–27 O4	S20–27	S13–27 O1

[a] A, August; S, September; O, October; N, November; F, February.
[b] Blooms reported: 15/11/78, 29/10/80, 27/10/81, 1/09/82, 4/10/83.
[c] ND, No data.

in sustaining the germination process. However, Theil and Wolk (25) showed the presence of substances in spent medium in which cultures had sporulated which completely suppressed akinete germination in *Nostoc* strains. The effects of chemical regulators on akinete germination have been examined by Yamamoto (27), who found that sodium acetate stimulated germination of *Anabaena* akinetes.

Rai and Pandey (16) found that omission of phosphate from the medium reduced germination of *Anabaena* to less than half. In the current work, *Nodularia* akinetes required small concentrations of phosphorus to germinate. Cyanobacterial akinetes appear to lack polyphosphate granules (cf. 12, 26). This is consistent with ultrastructural observations of *Nodularia* akinetes (A. L. Huber and J. Kuo, unpublished data). However, Talpasayi (24) suggested that the loss of metachromacity in developing akinetes (as observed by light microscopy) might only indicate the depolymerization of the long-chain polyphosphates, rather than their loss. Thus, from the literature, the true state of phosphate storage in akinetes is not quite clear. However, in the absence of polyphosphate granules, it is not likely that large amounts of excess phosphorus are stored. Therefore, the dependence of both akinete germination and outgrowth of the hormogonia on the presence of phosphorus seems logical.

Rai and Pandey (16) reported that nitrate induced early and maximum germination in *Anabaena vaginicola*. In its absence, germination was reduced to 42%. Conversely, Yamamoto (27) found no effect of nitrate on *Anabaena cylindrica* akinetes incubated in air and decreased germination in the presence of nitrate under anaerobic conditions. In the current studies, nitrate (to 800 μM) did not affect the germination rate of *Nodularia* akinetes. However, ammonia inhibited the germination rate at concentrations of >36 μM and completely suppressed germination at 153 μM. Cyanobacterial akinetes generally contain large numbers of cyanophycin granules, which serve as the primary nitrogen storage bodies (cf. 12, 23). This is true for *Nodularia* (Huber and Kuo, unpublished data) and, therefore, the lack of response of *Nodularia* akinetes to added nitrate is consistent. It is interesting to note that the *Anabaena* akinete cultures of Rai and Pandey (16) were prepared from cultures grown in the presence of ammonia. It could be speculated that this could account for some of the difference in response to nitrate by *Nodularia* and *Anabaena* akinetes. There are no other studies available for comparison.

The inhibition of germination of *Nodularia* akinetes by ammonia was not expected. If inhibition occurred in other species, it could function as a type of feedback inhibition, similar to that shown by Theil and Wolk (25) for spent medium.

Iron is required for the function of many enzymes, particularly nitrogenase. It is also a nutrient which may not always be biologically available even if it is present. In the current study, iron (chelated with EDTA) stimulated germination of *Nodularia* akinetes. To my knowledge, the effect of iron on germination of akinetes has not previously been reported.

Salinity of the Peel-Harvey Estuary water ranges from 2 to 51‰ (11); therefore, the controlling effect of salinity on *Nodularia* germination may be important. Germination was inhibited by salinities of >20‰ (0.34 M NaCl) and no germination occurred at 40‰. The only other study of the effect of NaCl on akinete germination is that of Pandey and Talpasayi (15), also on *Nodularia*, who used NaCl as a means of adjusting osmotic water potential. Percent germination was decreased by 24-h exposure to osmotic potentials of <-145 bars (172‰, 2.9 M NaCl) and by 96-h exposures to <-37 bars (45‰, 0.8 M NaCl). In their studies, germination rates were determined after the akinetes had been rinsed and plated onto agar, and therefore the ability of the akinetes to survive, rather than to germinate in high salt concentrations, was examined.

The effect of temperature on germination rates has been examined by Rai and Pandey (16) and Yamamoto (27). They found that 95 and 5% germination of *A. vaginicola* occurred at 25 and 35°C, respectively, and that pretreatment of akinetes in the dark at suboptimal temperatures increased the lag time for germination. Yamamoto (27) also found that the lag time decreased at 20 to 27°C incubation temperatures; no germination occurred at 35°C. In the current study, the temperature range examined was 12 to 24°C, since that was the range of interest with respect to estuary conditions. The germination rate of *Nodularia* akinetes increased, and the lag time decreased, with temperature to 22°C. These results are consistent with the previous studies.

Germination of *Anabaena* akinetes as a function of light intensity was examined by Braune (2), who showed an optimum intensity at between 5 and 20 W m^{-2}. Intensities of >25 W m^{-2} inhibited outgrowth. No germination occurred in the dark, but the lower limit of stimulation was only 0.1 to 0.3 W m^{-2}. Yamamoto (27) showed a decreasing lag time for germination with increasing intensity (100 to 3,000 lx, 0.16 to 4.83 W m^{-2}). Conversely, Kaushik and Kumar (9) reported no effect of light intensity on germination of *Anabaena doliolum* over the range from 30 to 200 lx (0.05 to 0.3 W m^{-2} for yellow light). The response of *Nodularia* to light intensity was similar to that observed by Braune (2), with increasing rates of germination from 0 to 9 microeinsteins m^{-2} s^{-1}. No germination occurred in the dark. The subsequent growth rate of *Nodularia* increased with increasing light intensities until light became inhibitory (80 microeinsteins m^{-2} s^{-1}); this is consistent with the reports of both Braune (2) and Kaushik and Kumar (9).

The most comprehensively examined factor affecting akinete germination appears to be light quality. All reports agree that only red light is necessary for germination (2, 9, 15–17, 27). Braune (2) showed that the red wavelengths (620 to 630 nm) corresponded to the maximum light absorption by C-phycocyanin. In the current study, *Nodularia* akinetes also required red light to germinate.

The minimum length of continuous exposure time to light required for germination of *Nodularia* akinetes was found to be 24 h of light at 9 microeinsteins m^{-2} s^{-1}.

If the above results are combined, a probable set of required conditions for rapid germination of *Nodularia* akinetes in the environment, in this case the Peel-Harvey Estuary, can be obtained. This, of course, is simplistic because interactions between parameters are likely to occur. However, these have not been examined. With respect to nutrients, small concentrations of phosphorus and iron should be available, nitrate need not be present, and ammonia concentrations should be <70 μM. With respect to physical conditions, the salinity should be <20‰, and the temperature should be above 16°C. The light intensity reaching the akinetes may be as low as 9 microeinsteins m^{-2} s^{-1}, but it needs to include red light and should reach the akinetes for the equivalent of more than 24 h of continuous light. The relationship between continuous light and light conditions at the sediment surface is not known. Further conditions required, but not tested here, are a pH range of 7.0 to 9.0 (16) and the presence of oxygen (27).

It appears that under natural conditions akinetes may require a combination of parameters to be within defined limits for germination to take place, and even a single parameter outside the suitable range may prevent germination. In the Peel-Harvey Estuary, the appropriate combination of conditions are usually met in the late spring and early summer, and, in the past 5 of 6 years, extensive *Nodularia* blooms have ensued.

ACKNOWLEDGMENTS

This work was supported by a research grant from the West Australian Department of Conservation and the Environment.

The help of D. K. Kidby and K. Hamel is gratefully acknowledged.

LITERATURE CITED

1. **Adams, D. G., and N. G. Carr.** 1981. The developmental biology of heterocyst and akinete formation in cyanobacteria. Crit. Rev. Microbiol. **9**:45–100.
2. **Braune, W.** 1979. C-phycocyanin—the main photoreceptor in the light dependent germination process of *Anabaena* akinetes. Arch. Microbiol. **122**:289–295.
3. **Braune, W.** 1980. Structural aspects of akinete germination in the cyananobacterium *Anabaena variablis*. Arch. Microbiol. **126**:257–261.
4. **Braune, W.** 1981. A study of akinete germination and the rhythmic longitudinal migration of young hormogonia in *Anabaena variablis* by means of time lapse photomicrography. Arch. Protistenkd. **124**:132–143.
5. **Grilli Caiola, M., and L. de Vecchi.** 1980. Akinete ultrastructure of *Nostoc* species isolated from cycad carralloid roots. Can. J. Bot. **58**:2513–2519.
6. **Huber, A. L.** 1984. *Nodularia* (*Cyanobacteriaceae*) akinetes in the sediments of the Peel-Harvey Estuary, Western Australia: potential inoculum source for *Nodularia* blooms. Appl. Environ. Microbiol. **47**:234–238.

6a. **Hughes, E. O., P. R. Gorham, and A. Zehnder.** 1958. Toxicity of a unialgal culture of *Microcystis aeruginosa*. Can. J. Microbiol. **4**:225–236.

7. **Jensen, T. E., and R. L. Clark.** 1969. Cell wall and coat of developing akinetes of a *Cylindrospermum* species. J. Bacteriol. **97**:1494–1495.
8. **Kappers, F. I.** 1976. Presence of blue green algae in sediments of Lake Brielle, p. 382–386. *In* H. L. Golterman (ed.), Interactions between sediments and freshwater. Proceedings of an International Symposium held at Amsterdam, The Netherlands. Dr. W. Junk B. V. Publishers, The Hague.
9. **Kaushik, M., and H. D. Kumar.** 1970. The effect of light on growth and development of two nitrogen fixing blue-green algae. Arch. Microbiol. **74**:52–57.
10. **Livingstone, D., and G. H. M. Jaworski.** 1980. The viability of akinetes of blue-green algae recovered from the sediments of Rostherne Mere. Br. Phycol. J. **15**:357–364.
11. **Lukatelich, R. J., and A. J. McComb.** 1981. The control of phytoplankton populations in the Peel-Harvey Estuarine System. Appendix 1. Tech. Rep. 93. Department of Conservation and Environment, Western Australia, Perth.
12. **Miller, M. M., and N. J. Lang.** 1968. The fine structure of akinete formation and germination in *Cylindrospermum*. Arch. Microbiol. **60**:303–313.
13. **Nichols, J. M., and D. G. Adams.** 1982. Akinetes, p. 387–412. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria. Blackwell Scientific Publications, Oxford.
14. **Nichols, J. M., and N. G. Carr.** 1978. Akinetes of cyanobacteria, p. 335–343. *In* G. Chambliss and J. C. Vary (ed.), Spores VII. American Society for Microbiology, Washington, D.C.
15. **Pandey, R. K., and E. R. S. Talpasayi.** 1981. Factors affecting germination of spores in a blue-green alga *Nodularia spumigena* Mertens. Acta Bot. Ind. **9**:35–42.
16. **Rai, A. I., and G. P. Pandey.** 1981. Influence of environmental stress on the germination of *Anabaena vaginicola* akinetes. Ann. Bot. **48**:361–370.
17. **Reddy, P. M., and E. R. S. Talpasayi.** 1981. Some observations related to red-far red antagonism in germination of spores of the cyanobacterium *Anabaena fertilissima*. Biochem. Physiol. Pflanz. **176**:105–107.
18. **Reynolds, C. S.** 1972. Growth, gas-vacuolation and buoyancy in a natural population of a blue-green alga. Freshwater Biol. **2**:87–106.
19. **Reynolds, C. S.** 1975. Interrelations of photosynthetic behavior and buoyancy regulation in a natural population of a blue-green alga. Freshwater Biol. **5**:323–338.
20. **Reynolds, C. S., and A. E. Walsby.** 1975. Water blooms. Biol. Rev. **50**:437–481.
21. **Roelofs, T. D., and R. T. Oglesby.** 1970. Ecological observations on the planktonic cyanophyte *Gleotrichia echinulata*. Limnol. Oceanogr. **15**:224–229.
22. **Rother, J. A., and P. Fay.** 1977. Sporulation and the development of planktonic blue-green algae in two Salopian meres. Proc. R. Soc. London Ser. B **196**:317–332.
23. **Sutherland, J. M., M. Herdman, and W. D. P. Stewart.** 1979. Akinetes of the cyanobacterium *Nostoc* PCC 7524: macromolecular composition, structure and control of differentiation. J. Gen. Microbiol. **115**:273–287.
24. **Talpasayi, E. R. S.** 1963. Polyphosphate containing particles of blue-green algae. Cytologia **28**:76–80.
25. **Theil, T., and C. P. Wolk.** 1982. Autoinhibition of spore germination in *Nostoc spongiaeforme* (Cyanophyceae). J. Phycol. **18**:305–306.
26. **Wildman, R. B., J. H. Loescher, and C. L. Winger.** 1975. Development and germination of akinetes of *Aphanizomenon flos-aquae*. J. Phycol. **11**:96–104.
27. **Yamamoto, Y.** 1976. Effect of some physical and chemical factors on the germination of akinetes of Anabaena cylindrica. J. Gen. Appl. Microbiol. **22**:311–323.